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An autoradiographic study of the intracellular transport of interstitial cell stimulating hormone in dissociated pituitary glands of normal and castrated rats

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An autoradiographic study of the intracellular transport of interstitial cell stimulating hormone in dissociated pituitary glands of normal and castrated rats

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INTRODUCTION

In the male rat, the gonadotrophic cells of the anterior pituitary gland secrete interstitial cell stimulating hormone (ICSH). This hormone is a glycoprotein and is thus a polypeptide with covalently linked carbohydrate moieties. The physiologic and biochemical effects of this hormone are mediated through cyclic adenosine 3'5'-monophosphate (AMP) which stimulates protein biosynthesis and steroidogenesis in the testis of the male rat. The production of ICSH is mediated by levels of ICSH-releasing hormone (ICSH-RH) in the hypothalamus which is in turn regulated by testosterone levels in the circulating blood. Castration removes this regulatory feedback mechanism and produces a hypertrophic condition in the organelle systems that synthesize and secrete ICSH in the gonadotrophs of the anterior pituitary.

The most common method for investigating secretory kinetics of the pituitary in vitro is to use intact tissue blocks in experimental and control preparations. With this system, studies of the secretory process at the subcellular level are limited by a number of problems. In the anterior pituitary there is a heterogeneous population of secretory cells (five types) plus endothelial and follicular cells which may be identified with the electron microscope. Compounding this heterogeneity problem is the difficulty encountered with the penetration of synthetic precursors (amino

acids, sugars, etc.) into tissue blocks.

The use of a system consisting of a suspension of single, dispersed pituitary cells in which cellular structure and function are retained essentially as in the in situ condition would avoid these difficulties by randomizing cell populations from a number of glands, eliminating diffusion and penetration problems and providing starting material for the subfractionation of these cell populations into preparations of single cell types.

Two techniques were used in this study to dissociate the anterior pituitary tissue of male rats. One procedure was that used by Hopkins and Farquhar (1973) which employs the subsequent incubation of tissue blocks in trypsin, DNAase, soya bean trypsin inhibitor, EDTA and neuraminidase followed by gentle shearing to disperse the tissue blocks. The other method, used by Portanova et al. (1970), utilizes mechanical agitation in trypsin with subsequent washing in lima bean trypsin inhibitor to obtain suspensions of isolated anterior pituitary cells.

Tritiated leucine and mannose were the labeled precursors used to visualize the synthesis, transport, storage and secretion of ICSH in the dissociated gonadotrophs found in these cell suspensions. Pulse labels of five minutes followed by incubation time intervals of 0, 5, 15, 30, 60, 120, and 240 minutes in cells from noncastrated, 15-day and 30-day castrated rats were employed to examine the secretory kinetics of this hormone.

REVIEW OF LITERATURE

The cells of the anterior lobe of the pituitary gland have proven to be less accessible to the cell biologist than those of other exocrine glands, such as the liver, parotid, and pancreas. Conventional fractionation and *in vitro* studies have proven successful for elucidating secretory mechanisms in the large homogeneous glands mentioned but have not been applicable to the anterior pituitary, with at least five small populations of cells whose secretory output is measured in microgram quantities rather than grams per day as in certain exocrine glands. Functions of the pituitary cells are less well-understood than other glandular cells. What is known has largely been obtained from morphological and localization techniques, i.e., light and electron microscopy, autoradiography, histochemistry, immunocytochemistry and enzyme cytochemistry, all of which may be carried out with intact tissues and, with the exception of autoradiography, give essentially static information.

In previous studies, numerous efforts have centered around attempts to establish functions and identities of the cellular types present. These studies have taken advantage of the negative feedback control that target endocrine organs impose on concomitant cell populations in the anterior lobe by target organ removal or target organ hormone therapy to stimulate or suppress, respectively, the production of a

particular trophic hormone. Available evidence indicates that a separate cell type exists for the production of each of these hormones with the exception of the gonadotropins; hence, the cells are named according to their secretory function. With the aid of light microscopy, the cells have been distinguished by differences in the stain affinity of their secretory granules. These staining properties have been reviewed by Herlant (1964) and Purves (1961, 1966). With the electron microscope, the single most important criterion for identifying cell types is the diameter of secretory granules. Initial identifications, except for the corticotrophs (Siperstein and Allison, 1965), were made as a result of electron microscopic and experimental studies carried out in the mid-1950's (Farquhar and Rinehart, 1954a,b; Hedinger and Farquhar, 1957). They were subsequently substantiated in morphological studies by others (Kurosumi, 1968; Pooley, 1971; and Costoff, 1973). Direct confirmation of these identifications came from immunocytochemistry (Nakane, 1970; Moriarty, 1973) and as a result of the isolation and characterization of the secretory granules (McShan and Hartley, 1965; Costoff and McShan, 1969; and Costoff, 1973).

Gonadotrophs

The gonadotroph has been known since the work of Purves and Griesbach (1951) and Halmi (1952) to be a large round

cell. It contains 200 nm secretory granules which are less osmiophilic than somatotrophs or prolactin granules (Kurosumi, 1968). The RER occurs characteristically in a vesicular form of various sizes and irregular forms with a content of greater density than that of the cytoplasmic matrix. After castration, the elements of the RER become progressively distended with nascent polypeptides and fuse so that individual cisternae become huge, producing the characteristic "castration" cell (Farquhar and Rinehart, 1954a,b). In addition to the 200 nm secretory granules, some gonadotrophs contain populations of 400 nm secretory granules (Hopkins and Farquhar, 1973). Immunocytochemical evidence (Tougard et al., 1973) shows that both types of granules contain secretory material which is stored gonadotrophic hormone. Within the community of gonadotrophic cells, morphologically distinct subtypes have been distinguished by electron microscopy (Kurosumi, 1968; Kurosumi and Oota, 1968; and Rennels et al., 1971) and by immunocytochemistry (Nakane, 1970; Moriarty, 1973; and Tougard et al., 1973). It has not been established whether one cell type is solely responsible for the formation of follicle stimulating hormone (FSH) and it is not clear whether both gonadotrophs are produced by the same or different cellular types. There is immunocytological evidence in the rat (Nakane, 1970) and in the human (Phifer et al., 1973) indicating the presence of both luteinizing hormone (LH) and FSH in the same cell; however, the studies cited were carried

out using antibodies raised to whole hormones which share a common polypeptide (alpha subunit). More recent studies with antibodies raised to the differentiating subunit (beta) (Baker et al., 1972; Tougard et al., 1973; Nakane, 1970; and Moriarty, 1973) have not resolved this problem thus far.

Somatotrophs

This is the most abundant cell type present in the anterior lobe, the growth hormone producing cell which can be readily identified by its generally ovoid or polygonal shape and the presence of a variable number of rounded or ovoid secretory granules approximately 350 nm in diameter (Kurosumi, 1968). These granules are intensely osmiophilic and appear uniformly dense. In young, growing animals this cell type contains a well-developed Golgi apparatus, a few lysosomes, and abundant rough endoplasmic reticulum (RER) consisting of stacks of elongated cisternae, with a content of low density. In somatotrophs of older animals, the RER and Golgi bodies are simpler, and many of the cells contain numerous lysosomes with heterogeneous content (Hopkins and Farquhar, 1973).

Mammotrophs

The mammotroph or prolactin cell has the largest secretory granules of any anterior lobe cell type. The mature granules are 600-900 nanometers (nm), ovoid or elliptical in shape, and homogeneously dense (Kurosumi, 1968). Immature

forms are smaller, and more variable in size and shape, due to the fact that they are formed by pinching off of smaller granules from the interior of the stacked Golgi cisternae, followed by a progressive aggregation of several small granules into polymorphous forms (Smith and Farquhar, 1966; Farquhar, 1969, 1971). Mammotrophs are the predominating cell type in the pituitaries of lactating females, less numerous but still abundant in pituitaries from cycling females, less frequent in pituitaries of male rats.

Thyrotrophs

The thyrotroph is typically angular in shape, and contains the smallest (140 nm) granules of any cell type. It is frequently said that thyrotrophs can be distinguished by the fact that their granules tend to line up beneath the cell membrane at the periphery of the cell; but, according to Nakane's (1970) immunocytochemical studies, this is not a feature specific for thyrotrophs since it is also found in corticotrophs. In the normal animal, the RER of thyrotrophs consists of a few simple, flattened cisternae of irregular distribution, but after thyroidectomy the RER becomes dilated and filled with a content of moderate density (Farquhar et al., 1975). The situation is therefore similar to that of the gonadotroph after castration, except that in the thyroidectomy cell intracisternal granules appear within the dilated RER cavities and become more numerous with increasing time

after thyroidectomy (Farquhar and Rinehart, 1954b; Kurosumi, 1968; and Farquhar, 1971).

Corticotrophs

A long and controversial history has preceded the identification of cells responsible for adrenocorticotrophic hormone (ACTH) production (Costoff, 1973). There is, however, a consensus emerging among interested researchers with converging evidence from electron microscopic studies of adrenalectomized rats by Siperstein and Allison (1965), Kurosumi and Kobayashi (1966), Rennels and Shiino (1968), Nakayama et al. (1969), Siperstein and Miller (1970), and Pelletier and Racadot (1971) and from immunocytochemical studies by Nakane (1970), Moriarty and Halmi (1972), and Bowie et al. (1973) indicating that this cell is characterized by 200 nm secretory granules and is typically angular in profile. In some cases, particularly following adrenalectomy, it has spiderlike arms radiating out from its cell body. It also has a simple RER consisting of sparse cisternae, abundant, free ribosomes, and a small Golgi apparatus. Nakane (1970) has noted a close association of these cells with prolactin cells.

Follicular Cells

These are nongranulated, presumably nonsecretory elements which were identified in early studies of the anterior pituitary by electron microscopy (Farquhar, 1957). Their

name stems from the fact that they line tiny ductules or follicles. These cells possess microvilli and a few cilia which project into the follicular lumen. They also have long slender, cytoplasmic arms which extend out between adjacent parenchymal cells. Junctional complexes join one cell to another near their luminal surface, but according to Vila-Porcile (1972), the junctions do not completely seal off the intercellular spaces since horseradish peroxidase, administered i.v., gains access to the follicular lumen. Follicular cells lack secretory granules but have large numbers of polysomes and beta particles of glycogen. They frequently contain lipid droplets and have small numbers of other cell organelles.

Secretory Process in Anterior Pituitary Cells

The information dealing with the secretory process in anterior pituitary cells is incomplete since it is based primarily on static techniques and is in part derived from work done on more accessible systems.

The anterior lobe of the pituitary gland produces six hormones, two simple proteins (growth hormone and mammotrophic hormone), three glycoproteins (thyrotropic stimulating hormone [TSH], FSH, and LH or ICSH), and a polypeptide (ACTH). Each of these hormones is produced by a separate cell type with the possible exception of the gonadotrophic hormones. Thus, within this gland, hormone secretion can be studied in

a number of different cellular types that exist side by side and produce secretory products with different chemical composition.

Protein synthesis and secretion

Current concepts of the sequence of intracellular events that take place during secretion, particularly the secretion of proteins, are based largely on the extensive work carried out on exocrine cells of the guinea pig pancreas by Palade and his co-workers (Palade, 1959; Palade, 1966; Palade et al., 1962; Jamieson and Palade, 1967a,b, 1968a,b,c, 1970; and Redman et al., 1966). By combining morphological and biochemical techniques (cell fractionation procedures, electron microscopy, and autoradiography), these authors have shown that protein secretion is a sequential multi-step process which proceeds as follows: the secretory products, i.e., the pancreatic digestive enzymes, are synthesized exclusively on bound polyribosomes attached to the membranes of the RER and immediately transferred across these same membranes into the cavities or cisternal spaces of the RER. From there, they are transported via small vesicles which pinch off the transitional elements of the RER to a condensing vacuole located in the Golgi region where concentration of the secretory product takes place, forming the mature secretory granule. Upon the appropriate stimulus, the granules are discharged into the acinar lumen by exocytosis, or fusion of the

granule membrane with the plasma membrane at the apical surface of the cell. Thus, the steps involved in the processing of digestive proteins are: synthesis, segregation, intracellular transport, concentration, storage and discharge. Work by Jamieson and Palade (1968a,c; 1971) has shown that intracellular transport and discharge are not coupled to protein synthesis, since they take place after protein synthesis is stopped with puromycin or cyclohexamide. They have also shown (Jamieson and Palade, 1968b; 1971) that intracellular transport from RER to condensing vacuoles, and granule discharge are dependent on the availability of respiratory energy since they are blocked by respiratory inhibitors (N_2, CN, antimycin A, oligomycin, and DNP) but are insensitive to glycolytic inhibitors (F, iodoacetate); on the other hand, the concentration step is not energy dependent, since it takes place in the presence of all these inhibitors.

Pioneering studies by Smith and Farquhar (1966) dealing with mammatrophs, protein secreting cells, depict the events of pituitary cell hormone secretion. There are two main variations in mammotroph secretion from the scheme worked out in the exocrine pancreas: (a) concentration of the secretory product takes place in the stacked Golgi cisternae (not in specialized condensing vacuoles, and (b) an intracellular mechanism exists for the disposal of undischarged secretory granules via lysosomes. In contrast to the situation in the pancreas, a discharge option exists: when cells are

stimulated to discharge their secretory product, the granules move toward and fuse with the cell membrane and are discharged in the usual manner by exocytosis. However, when secretion is suppressed, the granules move toward and fuse with lysosomes, a process given the name "crinophagy" as suggested by deDuve (1969).

Glycoprotein synthesis and secretion

From work on other systems, primarily the thyroid gland (Whur et al., 1969), it is known that conjugation of sugars during the synthesis of glycoproteins occurs both in the RER and in the Golgi complex, depending on the location of the sugar (proximal or distal) in the attached carbohydrate chain.

The peptide backbone of a glycoprotein is assembled on membrane bound polyribosomes. The first sugar may be incorporated into the peptide while it is still nascent on the polyribosome. Vectoral transport then delivers the peptide into the cisternal space of the RER (Schachter, 1973). The peptide is then transported through the channels of the endoplasmic reticulum from rough to smooth-surfaced membrane regions and eventually arrives at the Golgi apparatus. During this movement through the cellular membrane system, membrane-bound, multi-glycosyltransferase systems catalyze the step-wise transfer of monosaccharide prosthetic groups (Schachter and Roden, 1973). A glycosyltransferase catalyzes the transfer of a monosaccharide from a nucleotide-sugar

donor to a suitable acceptor. The physiological acceptor is usually a growing glycoprotein or glycolipid; however, some, but not all, glycosyltransferases can also transfer sugar to monosaccharides or small oligosaccharides (Schachter, 1973). A transferase is usually specific for a particular nucleotide sugar and these enzymes are therefore conveniently classified according to the sugar transferred, i.e., sialytransferase, galactosyltransferase, etc. No exception has been found to the rule that a single transferase catalyzes the synthesis of a single type of linkage.

Only a limited amount of information is available on the many factors that control the synthesis of glycoproteins. Whereas genes control the assembly of polypeptides by an accurate template mechanism, the synthesis of polysaccharide prosthetic groups is controlled by a nontemplate mechanism in which genes code for a large variety of glycosytransferases. In detailed studies (Rodén, 1970; Spiro, 1970) it was found that the transferases require a well-defined high molecular weight polypeptide as an acceptor and thus initiation of prosthetic groups appears to be controlled by the respective glycosyltransferase.

When initiation has occurred, elongation of the oligosaccharide is controlled primarily by the specificity of the multiglycosyltransferase system for acceptors. Every transferase provides the substrate for the next transferase (Gottschalk, 1972; Spiro, 1970). The factors that terminate

oligosaccharide chain elongation are unknown. Presumably termination occurs when the last glycosyltransferase (after sialyl or fucosyltransferase) in the multiglycosyltransferase system has acted on the growing glycoprotein molecule.

Function of glycoproteins

Carbohydrate prosthetic groups do not appear to play a role in the function of biologically active molecules such as enzymes and hormones. For example, Plummer and Hirs (1963, 1964) have shown that bovine ribonuclease A, which is devoid of carbohydrate, and bovine ribonuclease B, a glycoprotein, have similar enzymatic activities. The previously reported requirement for sialic acid residues in the biological activity of gonadotrophic hormones is now known to be due to more rapid clearance from the plasma of sialidase-treated hormone rather than to a role of sialic acid in the mechanism of hormone action (Van Hall et al., 1971a,b). Schachter (1973) has suggested that a possible role for protein-bound carbohydrate might be information storage by means of stereospecific diversity. Polysaccharides are rigid molecules with a high potential for structural diversity. Variations can occur in monosaccharide sequences, anomeric linkages between sugars (α or β), carbon positions to which the neighboring sugar is attached, branching, chain lengths, and chain initiation sites on the polypeptide backbone. It is therefore proposed that protein-bound oligosaccharide chains serve as

a language of communication between a molecule and a membrane or between two membranes.

Molecule-membrane interactions would include secretion phenomena. Eylar (1965) proposed that protein-bound carbohydrate serves as a passport or recognition signal which permits molecules destined for secretion to leave the cell. This theory is based on the observation that extracellular proteins are usually glycoproteins, whereas intracellular soluble proteins usually contain no carbohydrate. Unfortunately there are many exceptions to this rule; in particular, many secreted proteins don't have carbohydrate components. There is, however, evidence linking the Golgi apparatus, plasma membrane, glycosyltransferases, and secretion. If glycosylation is necessary for secretion, a mechanism must exist for subsequent removal of variable amounts of carbohydrate during or immediately after the secretory process; no such phenomena has yet been demonstrated. An alternative possibility is that glycosylation is required for movement of molecules across intracellular membrane barriers, e.g., for entry of glycoproteins into secretory vesicles. Such a concept would allow a longer time within the cell for removal of carbohydrate by intracellular glycosidases. Glycosylation may also be involved in controlling the movement of molecules destined for secretion through the membrane system of the cell while the nucleotide sugars may be a source of energy for this intracellular movement (Schachter and Roden, 1973).

In the male rat, the gonadotrophic cells of the adenohypophysis secrete ICSH and FSH. ICSH is structurally and chemically identical to LH, which is its analog in the female. Both of these hormones, ICSH (or LH) and FSH, are glycoproteins with two noncovalently bound dissimilar subunits designated α (common) and β (hormone specific) (Pierce et al., 1971). The β subunits determine the biological specificity of the respective hormones (Justiz and De La Rosa, 1972).

Steroidogenic effect of ICSH Hall (1966) has shown that ICSH increases the synthesis of testosterone and increases the mass of testosterone formed in rabbit testis. Moreover, contrary to previous findings, he states that ICSH increases the conversion of labeled cholesterol to testosterone in slices of rabbit testis in vitro. Instersitial cell stimulating hormone added in vitro or administered in vivo was without demonstratable effect upon the conversion of pregnenolone to testosterone by slices of testis. Thus, under the experimental conditions used, the hormone stimulates steroidogenesis at some step(s) beyond cholesterol and before pregnenolone. These findings have not indicated the exact site(s) in the biosynthetic pathway at which ICSH exerts its effect.

Adenyl cyclase activity As a result of studies which have dealt with the action of hormones on their target tissues, the concept has arisen that a number of hormones act as a two-messenger system. The first messenger in this concept

is the hormone or neurohormone which is released by stimuli which may be varied and complex. The first messenger travels to a receptor site on the surface of the effector cells and causes the activation of the enzyme adenyl cyclase within the membranes of the effector cells. This enzyme then catalyzes the conversion of ATP into cyclic 3', 5' AMP (cAMP) and inorganic pyrophosphate. The newly synthesized cAMP then interacts with a regulatory subunit that is part of a cAMP dependent protein kinase, freeing the catalytic subunit of the protein kinase to phosphorylate endogenous substrates (Bhalla, 1976).

Observations by Murad et al. (1969) have given support to the hypothesis that ICSH produces its steroidogenic effect in the corpus luteum and testis through increased formation of cAMP and ATP. Studies with ICSH in the corpus luteum (Marsh et al., 1966) have demonstrated the stimulatory effects of these hormones on cAMP accumulation. Furthermore, cAMP has been shown to mimic the steroidogenic effect of ICSH in the corpus luteum (Marsh and Savard, 1964). Adenyl cyclase activity has also been shown to be present in the testis (Sutherland et al., 1962). It seems probable that stimulation of steroidogenesis by ICSH is mediated through cAMP as it is in the corpus luteum. Sandler and Hall (1967) have reported that cAMP produces a steroidogenic effect like that of ICSH on the testis.

Thus, the two-messenger system for hormone action is apparently applicable to the action of ICSH which may

actually be a three-messenger system since the stimulation of testosterone formation in the testis by cAMP may be thought of as adding a third messenger to the system. ICSH has been shown to increase the levels of cAMP in its target tissues and cAMP has been shown to mimic the effects of ICSH in the tissues normally influenced by this hormone. Adenyl cyclase has been isolated and shown to be present in testicular tissue. These findings satisfy the conditions which Sutherland and Robinson (1967) set forth and this provides evidence that the steroidogenic effect of ICSH is mediated through the formation of cAMP in testicular cells.

The possible physiological effects of cAMP in the cell are varied. It may increase the number of activated enzymes within the cell, it may alter the permeability of the cell membranes, or it may initiate the synthesis of specific intracellular compounds.

Protein synthetic activity of ICSH Hall and Eik-Nes (1962) have shown that interstitial cell stimulating hormone increased the incorporation of labeled valine and labeled tryptophane into protein by slices of testis when the hormone was administered in vivo or added in vitro. This response was inhibited by chloramphenicol and puromycin. They also found that interstitial cell stimulating hormone did not stimulate the conversion of labeled cholesterol to labeled testosterone by homogenates of testis but reduced NADP was shown to increase this conversion. Slices of rabbit testis

were also shown to incorporate labeled acetate into labeled testosterone *in vitro*. These findings suggest that interstitial cell stimulating hormone stimulates protein synthesis by the testis and raises the possibility that this hormone may share with ACTH a mechanism of action based on increased provision of reduced NADP.

Irby and Hall (1971) have isolated Leydig cells from the testes of hypophysectomized rats and incubated them *in vitro* with ^{14}C labeled amino acids. ICSH was administered intraperitoneally as a single injection and increased the *in vitro* incorporation of ^{14}C labeled amino acids into Leydig cell protein. This increase was specific for ICSH and for the placental gonadotrophins HCG and pregnant mare serum (PMS); FSH was without effect. The response to ICSH was seen within 5 days of hypophysectomy and within 5 hours of injection of the hormone. Glucose was without effect upon amino acid incorporation into Leydig cell protein by cells from either intact of hypophysectomized rats. These results indicate that ICSH, administered *in vivo* to hypophysectomized rats, increases the incorporation of amino acids into protein by Leydig cells *in vitro*.

Cell surface receptor sites

Recent observations by Catt et al. (1972) have characterized gonadotrophin binding (receptor) sites which have a high affinity and specificity for LH and human chorionic

gonadotrophin (HCG). They have been demonstrated in subcellular fractions and homogenates of the rat testis and ovary and utilized for radioligand receptor assays and structure-function studies of these hormones. In these studies, LH and HCG have been shown to compete for binding to receptor sites in the rat testis and ovary. In addition, the terminal carbohydrate residues of these glycoprotein hormones are unnecessary for tissue binding (Catt et al., 1972) and subunits of the HCG molecule to possess little if any intrinsic binding inhibition activity in the dissociated form (Catt et al., 1972). Hormones other than LH and HCG cause no displacement of labeled HCG from the testicular binding sites (Catt et al., 1972). Thus, the properties of the gonadotrophin-luteinizing hormone binding sites present in rat testis homogenates are consistent with those of a specific hormone receptor with high affinity and low capacity for molecules bearing conformation characteristic of the biologically active sites of chorionic gonadotrophin and luteinizing hormone.

The binding of ^{125}I labeled chorionic gonadotrophin to rat testis is temperature dependent, with limited capacity (10^{-12} mole/g) and high affinity ($K_a = 2.4 \times 10M^{-1}$) for HCG at 24°C. Gonadotrophin binding is not significantly affected by variations in calcium concentration within the physiological range, and it is associated with membrane fragments during differential centrifugation of testis homogenates labeled

with ^{125}I chorionic gonadotrophin. These properties of the HCG/LH binding sites present in rat testis homogenate are consistent with those of a specific hormone receptor for gonadotrophin which activate testicular steroidogenesis.

Selective degradation of cAMP by phosphodiesterase

The two-messenger system which has been discussed previously for hormone action is apparently applicable to the action of ICSH and may represent a three-messenger system since the stimulation of testosterone formation in the testis by cAMP may be thought of as adding a third messenger to the two-messenger hypothesis. Interstitial cell stimulating hormone has been shown to increase the levels of cAMP in its target tissues and cAMP has been shown to mimic the effects of ICSH in the tissues normally influenced by the hormone. Adenyl cyclase, the enzyme associated with cellular membranes and reported to catalyze the conversion of ATP to cAMP has been isolated and shown to be present in testicular tissue. These findings satisfy the conditions which Sutherland and Robinson (1966) set forth and thus provide evidence that the steroidogenic action of ICSH is mediated through the formation of cAMP in testicular cells. Other findings indicate that cAMP is a versatile regulatory agent which acts to control a number of cellular processes.

Control of ICSH and FSH secretion

Secretion of ICSH and FSH by the anterior pituitary gland is controlled by a neurohormone released from the hypothalamic area and carried to its specific adenohypophyseal site of action by a portal blood system (Schally et al., 1968; McCann and Porter, 1969; Burgus and Guillemin, 1970; and Schally et al., 1971c.d.). This neurohormone has been isolated from porcine (Schally et al., 1971b.e.) and ovine (Amoss et al., 1971) hypothalami and is characterized as a decapeptide having the following structure: (Pyro) Glu-His-Try-Ser-Tyr-Gly-Leu-Arg-Pro-gly-NH_2 (Matsuo et al., 1971a.b.; Baba et al., 1971; and Burgus et al., 1971). Since this peptide stimulates the release of both LH and FSH under various experimental conditions (Schally, 1971a,b,c; Amoss et al., 1971), it has been called the LH-releasing hormone/FSH-releasing hormone or the gonadotropin-releasing factor (Matsuo, 1971; Monahan et al., 1971; and Geiger et al., 1971). Borgeat et al. (1972) have shown that synthetic gonadotrophin releasing factor elicits accumulation of adenophypophyseal cAMP and release of both LH and FSH which indicates that the neurohormone exerts its effects on cAMP by specific activation of adenylate cyclase in LH and FSH secreting cells rather than by inhibition of cyclic nucleotide phosphodiesterase.

Steroidal feedback systems

The gonad, regardless of whether it is an ovary or a testis, secretes androgens and estrogens. The adrenal gland also furnishes androgens to the systemic circulation. These prohormones are converted into an assortment of ring A reduced products in the central tissues (brain and pituitary). Naftolin and Ryan (1975) suggest that many central reproductive functions of these prehormone androgens may also require their conversion to estrogens. This central aromatizing activity is greater in males than in females and is affected by castration, sex steroid and antiandrogen pretreatment. The anterior hypothalamus is the most actively aromatizing central nervous tissue. The limbic system has also been found to convert androgens to estrogens. Thus, the central effects of androgens must be aromatization of androgens as well as ring A reduction.

Cellular receptors and mechanisms of action of steroidal hormones

A significant discovery in the study of the molecular process of steroid hormone action in the last decade was that of proteins that can selectively bind steroids in target cells. Many reports are now available to show that estrogen in the female or androgen in the male can be preferentially retained by the hypothalamus, an apparent site of hormone action in the regulation of gonadotrophin production or

sexual behavior (Eisenfeld and Axelrod, 1966; Kato and Villee, 1967; McEwen et al., 1970; Tuohimaa, 1971; Maurer and Wooley, 1971; Pérez-Palacios et al., 1973; Plapinger and McEwen, 1973; Pérez-Palacios et al., 1975; and Mahesh et al., 1975).

The distribution of androgen in concentrating neurons can be seen in specific areas of the brain by autoradiographic techniques (Sar and Stumpf, 1972). Selective retention of androgen occurs in areas of the preoptic parolfactory region, the hypothalamus, the hippocampus, and the amygdala as well as in the cells of the anterior pituitary. The topographic distribution of androgen in the brain agrees well with the areas that have been associated with the regulation of gonadotropin secretion and male sexual behavior (Sar and Stumpf, 1973a,b).

In the pituitary the nuclei of a small number of anterior lobe cells (about 15%) concentrate radioactivity one hour after testosterone-^{3}H injection (Liao, 1975). The cells of the intermediate and posterior lobes did not retain radioactivity. The labeling of anterior pituitary cells is confined to a 60% area of gonadotrophs (Sar and Stumpf, 1973a,b). However, estradiol-^{3}H in the male and female rats showed nuclear estrogen concentration not only in gonadotrophs but also in other areas of anterior pituitary cells (Stumpf, 1971). The differences in the topographic distribution of radioactivity in the brain and pituitary following treatment with ^{3}H steroids indicate that not all the action of androgen

in the pituitary is due to the conversion of testosterone to estrogen. These studies have shown that estrogen and androgen can act directly on the pituitary gland in addition to a negative feedback effect on the hypothalamus (Debeljuk et al., 1972). The quantity of receptors in the pituitary has been estimated to be sevenfold greater than in the hypothalamus. Hypothalamic receptors, however, are confined at the basal medial region and the concentration in this region (1.1 fmole/ug DNA) is similar to the concentration in the pituitary (1.3 fmole/ug DNA) (Anderson et al., 1973; Leavitt et al., 1973).

Studies on isolated sex steroid receptors in the hypothalamus and pituitary have been very limited, but both the cytoplasmic and nuclear fractions of these tissues appear to have estrogen receptor molecules similar to those in the uterus (Kahwanago et al., 1970; Clark et al., 1972; Payne et al., 1973; and Kato et al., 1974). Testosterone binding proteins are also found in the cytoplasm and in nuclear preparations of the anterior pituitary (Jouan et al., 1971). It has been shown that most of the protein-bound androgens can be identified as testosterone (Jouan et al., 1973). The formation of dihydrotestosterone from testosterone, however, occurs in the pituitary (Kniewald et al., 1970) or hypothalamus (Kniewald et al., 1971) and direct action of the dihydrotestosterone receptor complex is also possible.

Cytoplasmic nuclear interaction of steroid receptors

It is now clear that in most steroid target tissues, nuclear retention of steroids and receptors is dependent on prior formation of steroid-receptor complexes in the cytoplasm. This "two-step" mechanism was put forth independently by Jensen et al. (1968) and Gorski et al. (1968). There is a 4s receptor component in the cytoplasm which undergoes a steroid-dependent change in its conformation to become a 5s receptor component in the nucleus.

Chromatin receptor sites for receptors

The number of receptor binding sites in target cell nuclei has been estimated to be in the order of 2,000 to 10,000 per diploid genome, assuming one site for one receptor containing one steroid molecule (Liao, 1975); whether all these bindings represent biologically important interactions, is open to question. It is not clear whether more than one type of specific binding is involved in different functions in vivo. Studies in this area have been hindered by difficulty in distinguishing between specific and nonspecific binding.

Exocytosis

It has been suggested that hormone secretion occurs by fusion of the secretory granules with the cell membrane; this process is called exocytosis. It is sometimes stated or implied that exocytosis may not be the only or even the main

mechanism by which hormones are discharged from anterior pituitary cells. This discussion has centered in particular on the gonadotroph where images of exocytosis were not found (Kurosumi, 1968) even under conditions in which hormone discharge was stimulated by injection of hypothalamic extracts (Pelletier et al., 1971) or LH-RH (Rennels et al., 1971). Subsequently, however, exocytosis was observed by Shiino et al. (1972a) in LH gonadotrophs after administration of purified lutenizing hormone releasing factor (LRF or LH-RF) under conditions in which LH stores were reduced (i.e., androgen-sterilized, persistent-estrous rats). Hopkins and Farquhar (1973) incubated pieces of pituitary tissue from 10-day castrates in vitro in the presence of LRF (1.6 ng/ml for 1-30 minutes) and examined them by electron microscopy. They found images of exocytosis, but these were restricted to only a few of the gonadotrophs present. These findings indicate that discharge occurs by exocytosis, but only a few of the gonadotrophs present are responsive to LRF under these conditions.

In the case of anterior pituitary cells, there is good morphological evidence for exocytosis in the case of the somatotroph (Farquhar, 1961a,b; De Virgiliis et al., 1968; Couch et al., 1969; and Pelletier et al., 1972), the mammotroph (Pasteels, 1963; Smith and Farquhar, 1966, 1970; Pelletier et al., 1971, 1972; and Shiino et al., 1972b), the thyrotroph (Farquhar, 1969, 1971; Pelletier et al., 1971;

Shiino et al., 1973; and Moguilevsky et al., 1973), the corticotroph (Rennels and Shiino, 1968; and Pelletier and Racadot, 1971) and, as mentioned above, even the gonadotroph (Shiino et al., 1972a; and Hopkins and Farquhar, 1973).

It has been suggested that in the anterior pituitary (Sherline et al., 1977) as well as in other secretory cells (Pelletier and Bornstein, 1972; Labrie et al., 1973; and Kracier and Milligan, 1973), a microtubule-microfilament system may be involved in exocytosis. The frequency of microtubules or microfilaments in pituitary cells is not overwhelming, and there appears to be no special association of of such structures with secretory granules at the periphery of the cell.

The involvement of these structures is postulated on the basis of indirect evidence, i.e., that drugs, such as colchicine and vincristine (which bind to microtubule protein) and cytochalasin B (which is supposed to affect microfilaments) inhibit secretion in various systems. This hypothesis is open to question for several reasons: cytochalasin B interferes with all energetics by inhibiting glucose uptake (Zigmond and Hirsch, 1972; and Mizel and Wilson, 1972a). Colchicine and vincristine depress ATP production in some (Jamieson, 1972) but not all cells (Le Marchand et al., 1973). Therefore inhibition of secretion may be explained in some cases by a reduction in intracellular ATP concentrations. Colchicine also binds to cell membranes (Stadler and Franke, 1974); and colchicine, together with several of its analogs

(colcemid and lumicolchicine), drastically inhibits nucleoside uptake in certain cells (Mizel and Wilson, 1972b). Therefore it remains to be decided by future work whether or not the drugs mentioned above inhibit secretion by interference with microtubules and microfilaments or by direct interaction with the cell membrane.

Crinophagy

In 1966, it was demonstrated that in pituitary mammotrophs, lysosomes play a role in regulating the secretory process by disposing of undischarged hormone (Smith and Farquhar, 1966). When hormonal secretion was suppressed, excess secretory granules were taken up and disposed of by lysosomes, a process given the name "crinophagy" by deDuve (1969). This mechanism was found to exist in somatotrophs, gonadotrophs, and thyrotrophs (Farquhar, 1969, 1971). This control mechanism, in contrast to those described above, is a secondary level control mechanism which operates presumably when there is an overproduction of secretory products. Pituitary lysosomes are known to contain proteases and peptidases capable of successively degrading pituitary hormones down to the level of inactive oligopeptides and dipeptides (McDonald et al., 1971). Since the latter can diffuse through the lysosomal membrane into the cell sap, they will presumably be further degraded into their constituent amino acids which are thereby restored to the metabolic pool for

neutralization within the pituitary cell (Farquhar, 1971; and McDonald et al., 1971).

Dissociation

Our knowledge of the secretion and action of hormones has been derived in large measure from studies on the whole animal, the perfused organ, the tissue slice, and the tissue homogenate. Isolated cells of the endocrine gland represent a new approach to such studies.

A number of successful attempts have been made to produce viable single cell suspensions from a variety of tissues, e.g., liver (Howard et al., 1967; and Berry and Friend, 1969), thyroid (Maayan and Ingbar, 1968), adrenal (Sayers et al., 1971; Halkerston et al., 1968; and Swallow and Sayers, 1969), corpus luteum (Gospodarowicz and Gospodarowicz, 1972), and exocrine pancreas (Amsterdam and Jamieson, 1972), including the anterior pituitary (Portanova et al., 1970; Hymer and Evans, 1970; Bala et al., 1970; Sayers et al., 1971; Vale et al., 1972; Ishikawa, 1969; Leavitt et al., 1973; Hopkins and Farquhar, 1973; Hymer et al., 1973; and Nakano et al., 1976). Most of these procedures have involved the use of collagenase, hyaluronidase, trypsin, pronase, EDTA, calcium-free media and neuraminidase in the case of Hopkins and Farquhar (1973). It appears essential that an initial protease treatment be used and this is in agreement with a recent study by Amsterdam and Jamieson (1972). Crude

proteolytic enzyme extracts or proteases other than trypsin, e.g., Viokase (Vale et al., 1972) and pronase (Leavitt et al., 1973) have been used for pituitary cell dispersion, but none has the dual advantage of being well-characterized and of having available a selective inhibitor such as lima bean trypsin inhibitor (LBTI) which is effective under physiological conditions. A number of investigators have emphasized the importance of specifically inhibiting protease activity, since these enzymes, which are known to adsorb to the cell surface and survive conventional washing procedures (Barnard et al., 1969; Wallis et al., 1969; and Poste, 1971) can cause injury to the cell surface and prevent reappearance of the cell coat material removed by the enzyme action even at very low concentrations (Barnard et al., 1969; and Poste, 1971). However, when protease treatment is brief and efficiently terminated with appropriate inhibitors, removal of cell coat material is reduced and resynthesis and replacement of these cell surface components occur within hours (Zajac and Crowell, 1965; Schwartz and Nathenson, 1971; and Kono, 1969). Thus dissociation of anterior pituitary tissue with trypsin followed by a trypsin inhibitor is the method which yields the greatest number of cells with minimal alteration of cell surface moieties.

Autoradiography

Since the first introduction by Liquier-Milward (1956), electron microscope autoradiography of biological specimens has become an established tool for tracing events within cells. The history, theory, and preparatory procedures as well as practical applications of this method have been extensively reviewed by various authors in recent years (Pelc, 1963, 1972; Caro, 1962, 1964; Bachmann and Salpeter, 1965; Salpeter and Bachmann, 1965; Granboulan, 1963, 1965; Salpeter, 1966; Kopriwa, 1966; Rogers, 1967; Hülser and Rajewsky, 1968; Bachmann and Salpeter, 1971; Vrensen, 1970; Williams, 1969; Whur, Herscovics and Leblond, 1969; Jacob, 1971; Budd, 1972; Sanderson, 1975; and Burry and Lasher, 1975).

The size of the undeveloped silver halide crystals and the thickness of the emulsion are among the limiting factors in autoradiographic resolution. Ideally, one wants a fine-grained emulsion coated in a closely packed monolayer. The Ilford L4 nuclear track emulsion with a silver halide grain size of 1200 to 1600 Å is most frequently used for electron microscopic autoradiography. A monolayer of Ilford L4 would be 1500 Å thick and have a purple interference color.

In recent years, there has been a lively debate whether the technique should be called autoradiography or radioautography. Leblond points out that the term radioautography is preferable because autoradiography means a positive picture obtained when the object under investigation is itself the

source of the energy, and thus radioautography would remain a self-produced image by means of radiation. On the contrary, radiography has come to mean a negative image produced when the object under investigation is located between the source of radiation and the emulsion. Webster's Third International Dictionary favors the term autoradiography, probably on the basis of its broader definition of radiograph as a picture produced upon a sensitive surface by a form of radiation other than light. Since, in the same dictionary, the prefix aut- is also defined as self-caused or self-induced, autoradiography can be understood as a self-caused picture produced by forms of radiation other than light. It is difficult to decide between these two arguments; but in deference to Lacassagne and Lattes (1924a) who coined the term "autoradiographic" and were among the foremost pioneers in the field, the term "autoradiography" should be generally adopted.

Hopkins and Farquhar (1973) established that suspensions of pituitary cells are capable of incorporating leucine-^{3}H into somatotrophic hormone at a rate 90% greater than that of tissue blocks. Thus pulse-labeling of cell suspensions, and subsequent electron microscopic autoradiography, appeared to provide the most efficient means to assess the cell's ability to transport and concentrate secretory products and to determine the intracellular route taken by newly synthesized glycoprotein over a period of time (4 hours).

The intracellular pathway of newly synthesized secretory product in dissociated somatotrophs (Hopkins and Farquhar, 1973) is essentially the same as that described in other protein secretory cells, especially those of the exocrine pancreas (Jamieson and Palade, 1967a,b), parotid (Castle, Jamieson and Palade, 1972), pituitary pars intermedia (Hopkins, 1972), beta cells of the pancreatic islets (Howell et al., 1969), and prolactin cells (mammotrophs) of the anterior pituitary (Tixier-Vidal and Picart, 1967; and Meldolesi et al., 1972). Information on the timetable of events in the secretory process of somatotrophs from intact tissue comes from a paper by Racadot et al. (1965) who carried out an in vivo autoradiographic study, and from Howell and Whitfield (1973) who combined cell fractionation and autoradiography to investigate growth hormone secretion by pituitary tissue incubated in vitro. Hopkins and Farquhar (1973) obtained their results by pulse-labeling cells in a suspension. Their findings were similar to those obtained previously in undissociated tissue and to the more detailed data available for the other secretory systems cited above. In all cases, there is relatively rapid (5-15 minutes) transport of secretory product to the Golgi complex followed by less rapid (20-60 minutes) transport to secretory granules. Thus, the time interval needed for traversal of the intracellular secretory pathway, in dissociated protein secreting cells as indicated by autoradiography, is in agreement with previous estimates (Labrie et al.,

1971) of 2 hours derived by following the appearance of labeled, newly synthesized, somatotrophic hormone in the medium, where hemipituitaries were incubated.

The only information available for glycoprotein secretory cells comes from a study done by Whur et al. (1969) in which galactose and mannose-^{3}H were incorporated into thyroglobulin using intact rat thyroid lobes. Their results show that mannose-^{3}H localizes within 5 minutes in the RER and by 1-2 hours, most is transferred to the Golgi complex. At 3 hours, significant numbers of silver grains were found over apical vesicles and colloid. Galactose-^{3}H was localized initially over the Golgi apparatus and subsequently transferred to the apical vesicles at a rapid rate, before movement to the colloid. Thus, mannose is incorporated into thyroglobulin within the RER. These precursors then migrate to the Golgi apparatus where galactose is added. The glycoprotein migrates via the apical vesicles to the colloid.

The application of autoradiographic techniques to the study of glycoprotein synthesis, secretion, and storage in gonadotropic cells of the anterior pituitary will delineate location and time parameters, thereby elucidating secretory product transport.

MATERIALS AND METHODS

Two techniques were evaluated for dissociating the pituitary tissue. The first was that of Hopkins and Farquhar (1973); the second was the technique Portanova et al. (1970) had used. The method used to dissociate the tissue in this study employs some procedures from each of the studies mentioned above.

Chemicals

Reagents were obtained from the following sources: bovine pancreatic trypsin (type 111, 2x crystalized) and essential amino acids (Eagle, 1959) were purchased from Sigma Chemical Company (St. Louis, Mo.). Chromatographically pure neuraminidase (Clostridium perfringens) and deoxyribonuclease I (RNAase-free) were purchased from Worthington Biochemical Corporation (Freehold, N.J.). Bovine serum albumin (fraction V) was purchased from Miles Laboratories, Incorporated (Kankakee, Ill.). Glutaraldehyde (50%), propylene oxide, epon components, uranyl acetate and lead citrate were purchased from Fisher Chemical Company (Chicago, Ill.), osmium tetroxide from Stevens Metallurgical Corporation (New York, N.Y.), tritiated leucine and mannose (1m ci/ml) from New England Nuclear (Boston, Mass.), 95% O_2 - 5% CO_2 from Matheson Scientific (Chicago, Ill.), and Ilford L-4 liquid emulsion from Polysciences (Warington, Pa.).

Animals

The animals used in this work were 250-300 gram Sprague Dawley male rats obtained from a breeding colony in the Department of Zoology, Iowa State University. The animals were castrated by ligature of the testes for 15 and 30 days duration. One female rat, 16 days pregnant, was also used.

Incubation Media

The incubation medium used throughout this study consisted of a Krebs-Ringer bicarbonate (KRB) solution (Krebs, 1950) containing a complete amino acid supplement, 0.3% bovine serum albumin (BSA), 2 mM glutamine pyruvate, fumarate, glutamate and glucose. The incubation solution, minus the amino acid supplement, consisted of the following components:

80 parts 0.154 M NaCl

4 parts 0.154 M KCl

3 parts 0.11 M $CaCl_2$

1 part 0.154 M KH_2PO_4

1 part 0.15 M $MgSO_4 \cdot 7H_2O$

21 parts 0.154 M $NaHCO_3$ (gassed with CO_2 until pH is 7.4)

4 parts 0.16 M Na-pyruvate

7 parts 0.10 M Na-fumarate

4 parts 0.16 M Na-glutamate

2 mM (ℓ) glutamine

5 parts 0.3 M glucose

3% bovine serum albumin (BSA)

The last four components are prepared by neutralizing a solution of the acids with 1.0 M $NaHCO_3$. The entire mixture is then saturated with a gas mixture containing 95% O_2 - 5% CO_2. The amino acid supplement which was added to the media listed on the previous page consisted of the following components (ℓ form except where noted) in the final concentration:

0.6 mM arginine
0.1 mM cystine
2.0 mM glutamine
0.2 mM histidine
0.4 mM isoleucine
0.4 mM leucine
0.4 mM lysine
0.1 mM methionine
0.2 mM phenylalanine
0.4 mM threonine (dl)
0.05 mM tryptophan
0.2 mM tyrosine
0.4 mM valine (dl)

For the Hopkins and Farquhar technique, a calcium and magnesium-free Krebs Ringer bicarbonate (KRB) medium with supplements listed above and on the previous page was prepared as follows:

83 parts 0.9% NaCl
4 parts 1.15% KCl
1 part 2.11% KH_2PO_4
3 parts 1.3% $NaHCO_3$

18 parts Na-PO_4 buffer (100 parts 0.1 M Na_2HPO_4 and 25 parts 0.1 M NaH_2PO_4)

4 parts 0.16 M pyruvate

7 parts 0.1 M Na-fumarate

4 parts 0.16 M Na-l-glutamate

5 parts 0.3 M glucose

A low-calcium and low-magnesium incubation solution was prepared by using 0.1 mM $CaCl_2$ and 1.2 mM $MgSO_4 \cdot 7H_2O$ in place of the higher concentrated solutions in the normal medium. The complete amino acid supplement was added to all of these media.

Dissociation of Tissue

Hopkins and Farquhar technique

All of the glassware was siliconized in a 1:1000 solution of siliclad, and all of the incubation media were millipore filtered (0.45 μm filter) before use. Thirty animals were decapitated and their pituitaries removed for each experiment; the posterior and intermediate lobes were lifted away with a forceps, and each anterior lobe was chopped into approximately 50-60 tissue blocks. The tissue blocks were pooled in a 50 ml Erlenmeyer flask containing 20 ml KRB with 0.3% BSA added at room temperature. The blocks were transferred to 15 ml conical centrifuge tubes and allowed to settle out. The KRB/BSA was decanted and replaced with 5 ml KRB/BSA containing 1 mg/ml trypsin at 25°C. The blocks and trypsin medium were transferred to a 25 ml Erlenmeyer flask

and incubated for 15 minutes at 37°C in a shaker bath 65-70 strokes/minute under an atmosphere of 95% O_2 and 5% CO_2. After the trypsin incubation, 5 ml of DNAase (2 µg/ml) in KRB/BSA was added to the flask and the contents poured into a conical centrifuge tube. The DNAase treatment was continued until the tissue blocks fell freely to the bottom of the tube (about one minute). The supernatant was decanted and replaced with 5 ml of prewarmed KRB/BSA containing 1 mg/ml soya bean trypsin inhibitor which was incubated for 5 minutes at 37°C. The supernatant was decanted and replaced with 5 ml of calcium and magnesium-free KRB/BSA containing 2 mM EDTA; this was incubated for 5 minutes at 37°C. The supernatant was decanted and replaced with 4 ml of calcium and magnesium-free KRB/BSA containing 1 mM EDTA and 8 µg/ml neuraminadase. The blocks and medium were transferred to a 25 ml Erlenmeyer flask and incubated in a shaker bath for 15 minutes at 37°C under an atmosphere of 95% O_2 and 5% CO_2. Following this incubation, the blocks were transferred to a conical centrifuge tube and washed three times with 20 ml of calcium and magnesium-free KRB/BSA by a settling out and decantation procedure to dilute the EDTA and neuraminadase. A flame polished Pasteur pipette was used to shear the tissue blocks. Milky suspensions of cells were decanted into a tube containing KRB with 0.1 mM $CaCl_2$ and 1.2 mM $MgSO_4$ and 5 mg/ml BSA. More calcium and magnesium-free KRB/BSA was added to the remaining tissue blocks, and the shearing was continued until

dissociation was complete. To remove debris, the cell suspension was centrifuged through BSA as follows: 5 ml of 4% BSA in KRB was layered below the cell suspension using a hypodermic syringe with a five-inch needle. The tube was centrifuged for 9 minutes at 70Xg producing a cell pellet. The debris was decanted and the cells resuspended in low calcium and magnesium KRB with 5 mg/ml BSA added.

These cells were fixed for 30 minutes at 25°C by adding an equal volume of 3% glutaraldehyde in 0.1 M phosphate buffer with 4% sucrose. The suspensions of fixed cells were then transferred to 0.4 ml polyethylene tubes, and the cells were packed into pellets by centrifugation at 10,000Xg in a Beckman microfuge 152 for 4 minutes. The tips of these tubes were cut off, and the pellets were removed by flushing with a Pasteur pipette (Bainton and Farquhar, 1968). These pellets were post fixed for one hour at 4°C in 1% osmium in 0.1 M veronal acetate buffer (pH 7.4) with 5% sucrose added. They were then stained en bloc by immersion in 0.5% uranyl acetate in veronal acetate buffer with 4% sucrose at room temperature for one hour. They were washed in veronal acetate buffer with 4% sucrose, dehydrated in graded ethanols and propylene oxide then embedded in epon.

Intact tissue blocks were trimmed to a pyramid with a razor blade or an LKB pyramitome. Sections 2 microns thick were cut using glass knives and were stained with Azure II to locate desirable areas of the block which were to be

thin sectioned. Silver-grey sections were cut with a diamond knife on either an LKB III or a Reichert OMU-2 ultramicrotome. Ribbons of sections were collected on 75x300 copper mesh grids and stained with 5% aqueous uranyl acetate and Reynold's (1963) lead citrate for viewing in the electron microscope.

The embedded pellets of cells were trimmed by hand with a razor blade or with an LKB pyramitome. Thick sections were cut with glass knives and stained with Azure II to find suitable portions of the block to be sectioned with the ultramicrotome. Silver-gold sections were cut with a diamond knife on either an LKB III or a Reichert OMU-2 ultramicrotome. These sections were collected on 75x300 copper mesh grids and either stained with a 5% aqueous uranyl acetate and Venable and Coggeshall's (1965) lead citrate or Reynold's (1963) lead citrate for viewing in the electron microscope or they were prepared for autoradiography, as described below.

Portanova, Smith and Sayers technique

For this technique, 0.25% trypsin was added to KRB to make KRBT (trypsin medium). Trypsin inhibitor (KRBI) was prepared by mixing the following components:

12 ml KRB (pH 7.4)

0.3% BSA

0.2% glucose

32 mg lima bean trypsin inhibitor

All of the media were gassed with 95% O_2 - 5% CO_2 (to pH 7.4), and this atmosphere was maintained either by intermittent gassing at 20-minute intervals or by continuous gas flow. All glassware was siliconized, and all media were millipore filtered through 0.45 μm filters before use.

The pituitaries were obtained as above. The tissue blocks were pooled in a 50 ml siliconized Erlenmeyer flask containing 20 ml of KRBT (trypsin media) at 25°C and incubated for 60 minutes at 37°C. During the incubation, the tissue blocks were agitated by a magnetic stirring bar and gassed with 95% O_2 and 5% CO_2. The cells were drawn off at 20-minute intervals and kept in an iced siliconized 250 ml Erlenmeyer flask. Twenty ml of KRBT was added after the aliquot of cells was removed. The combined cell suspensions were decanted into two 50 ml centrifuge tubes and centrifuged at 100 g for 30 minutes. The supernatant was decanted and the cells were resuspended in 12 ml KRB/BSA (3%) with 32 mg lima bean trypsin inhibitor added.

The cells were fixed, stained, dehydrated, and embedded as in the procedure described for the Hopkins-Farquhar technique.

Dissociation technique used in this study

The pituitary glands were obtained as in the previous methods. They were minced and added to a 50 ml Erlenmeyer flask (siliconized) containing 20 ml of KRBT which was

prepared as follows:

20 ml KRB buffer, complete amino acid supplement

0.2% glucose

0.25% trypsin

pH 7.35 to 7.40

gassed ten minutes with 95% O_2 and 5% CO_2

The tissue was agitated for 60 minutes at 37°C with a siliconized glass paddle at 500 rpm in an atmosphere of 95% O_2 and 5% CO_2. Dissociated cells were drawn off at 10-minute intervals into an iced siliconized 250 ml Erlenmeyer flask. Additional KRBT was added to the undissociated tissue after each interval. Two ml of rat serum and 1% glutamine were added to the suspension of cells after each aliquot of dissociated cells was collected. After the dissociation of the tissue the cell suspension was divided equally into two 50 ml centrifuge tubes and the cells were prepared for the incorporation of isotopes.

Incorporation of isotopes

The cells were centrifuged for 30 minutes at 7000Xg in a Beckman J21 centrifuge at 4°C. The supernatant was decanted and discarded. The pellets were resuspended in KRBI (lima bean trypsin inhibitor medium) with 15% rat serum and centrifuged again as described. The supernatant was decanted and discarded. The pulse labeling medium was prepared by mixing the following components:

2.0 ml minimal Eagles (pH 7.4) with 10% rat serum

2.0 ml KRBI (pH 7.4)

0.5 ml (ℓ) leucine-^{3}H (16.8 μmoles) or (d) mannose-^{3}H (377.4 μmoles) with 60 Ci/mM and 13.2 Ci/mM activity respectively

The Chase medium was prepared by mixing the following components:

2.0 ml minimal Eagles solution (pH 7.4) with 10% rat serum

2.0 ml KRBI (pH 7.4)

2 mM leucine or mannose

In the experiments with mannose-^{3}H, sodium pyruvate was added to all media to a concentration of 10 mM to reduce the conversion of this substrate into amino acids (Herscovics, 1969). The pellets were resuspended in 4.5 ml of label. The cells were allowed to incubate for 5 minutes at 37°C. At the end of this incubation period, a 0.5 ml aliquot was taken and fixed for the 0-minute sample, four ml of chase was added immediately and a 0.5 ml aliquot was taken at 5 minutes. The remaining suspension was centrifuged at 7000Xg for 10 minutes. The supernatant was decanted and discarded. The pellet was resuspended in 5.5 ml of minimal Eagles medium containing 15% serum and 1% glutamine. A 1.0 ml aliquot was taken and fixed for the 15-minute sample. The remaining cells were transferred to a 25 ml siliconized Erlenmeyer flask and incubated at 37°C in a Dubnoff shaker bath. One ml

aliquots were taken at 30, 60, 120, and 240 minutes and fixed.

The suspensions of fixed cells were decanted into plastic 0.4 ml microfuge tubes and centrifuged at 10,000 g for 4 minutes. The tips of these tubes were cut off; the pellets of cells were removed and processed for electron microscopy, as described above.

Autoradiography

Copper grids (75x300 mesh) with silver-gold sections were coated with Ilford L4 emulsion by the following technique: The grids with unstained sections were attached to glass slides using double-stick tape. A thin carbon coat was evaporated onto the sections. In a darkroom under a #2 Wratten safelight, four grams of Ilford L4 emulsion were melted in 40 ml of glass distilled water at 60°C. The mixture was stirred until smooth. The emulsion was allowed to cool for 30 minutes in an ice bucket. The grids attached to the slides were coated by stretching the emulsion into a thin film with an adjustable loop made of stainless steel wire. The slides were allowed to dry vertically and placed in a black slide box with a desiccant; the box was sealed with black tape and placed in a light tight container. The emulsion was exposed in the refrigerator for a minimum of six months. The slides were developed with Microdol X for 3 minutes, washed, fixed for 4 minutes, washed thoroughly, and dried. All photographic solutions were millipore filtered prior to use.

The grids were stained with uranyl acetate and lead citrate and examined in the electron microscope.

Electron microscopic observations were performed with an Hitachi HU11E electron microscope operated at 50 KV with a 30 μm objective aperture or with a Hitachi HU12A electron microscope operated at 50 or 75 KV with a 50 μm objective aperture.

List of Terms

The terminology used in this dissertation is defined as follows:

AMP – adenosine monophosphate.

ACTH – adrenocorticotropic hormone.

ATP – adenosine triphosphate.

BSA – bovine serum albumin.

CAMP – cyclic adenosine monophosphate.

CN – ionic cyanide.

DNAase – deoxyribonuclease.

DNP – dinitrophenol.

EDTA – ethylenediamine tetraacetic acid.

ER – endoplasmic reticulum.

F – ionic fluorine.

FSH – follicle stimulating hormone.

HCG – human chorionic gonadotropin.

^{125}I – isotope of iodine.

ICSH – interstitial cell stimulating hormone.

ICSH-RH – interstitial cell stimulating hormone-releasing hormone.

KRB – Krebs-Ringer bicarbonate.

KRBI – Krebs-Ringer bicarbonate with trypsin inhibitor added.

KRBT – Krebs-Ringer bicarbonate with trypsin added.

LBTI – lima bean trypsin inhibitor.

LH – luteinizing hormone.

LH-RH – luteinizing hormone-releasing hormone.

LRF – luteinizing hormone releasing factor.

M – molarity of a solution.

mCi – millicurie (10^{-3} currie).

mM – millimolarity (10^{-3} molar).

N_2 – nitrogen gas.

NADP – nicotinamide adenine dinucleotide.

nm – nanometers (10^{-9} meters).

RER – rough endoplasmic reticulum.

RNAase – ribonuclease.

SBTI – soya bean trypsin inhibitor.

SER – smooth endoplasmic reticulum.

TSH – thyroid stimulating hormone.

μ – micron (10^{-6} meters).

μg – microgram (10^{-6} grams).

μm – micrometer (10^{-6} meters).

RESULTS

The results can be divided into two sections: (1) feasibility of dissociating castration cells and (2) study of gonadotrophin synthesis.

Feasibility of Dissociating Castration Cells

The ultrastructural preservation of the different pituitary cell types both in intact and dissociated tissue following 0, 15, and 30 days of castration was examined.

Normal cells (intact tissue)

Untreated rat anterior hypophyses were characterized by the presence of many capillaries with characteristic endothelial cells, red blood cells, and platelets (Figures 1 and 2). Surrounding the capillaries is a space which may contain collagen fibers (Figure 2). Occasionally cilia are observed in intact tissue (Figure 3). All five of the cell types as described by Kurosumi (1968) were observed.

Gonadotrophs The gonadotrophs were large (up to 16 μ in diameter), round cells and often occurred along blood capillaries (Figures 1, 2 and 3). These cells contained many secretory granules which were classified into two groups, one larger (400 nm) in diameter and less dense than the other more abundant smaller (200 nm), dense, granules (Figures 1, 2, 3, 6 and 7). Most of the mitochondria were rod-like but a few were spherical (Figures 6 and 7). Most of the

Figure 1. Intact adenohypophysis showing relationship of: gonadotrophs (GT), somatotrophs (ST), capillary (Cap), red blood cells (Rbc), endothelial cell (E) and platelets (P). x5200.

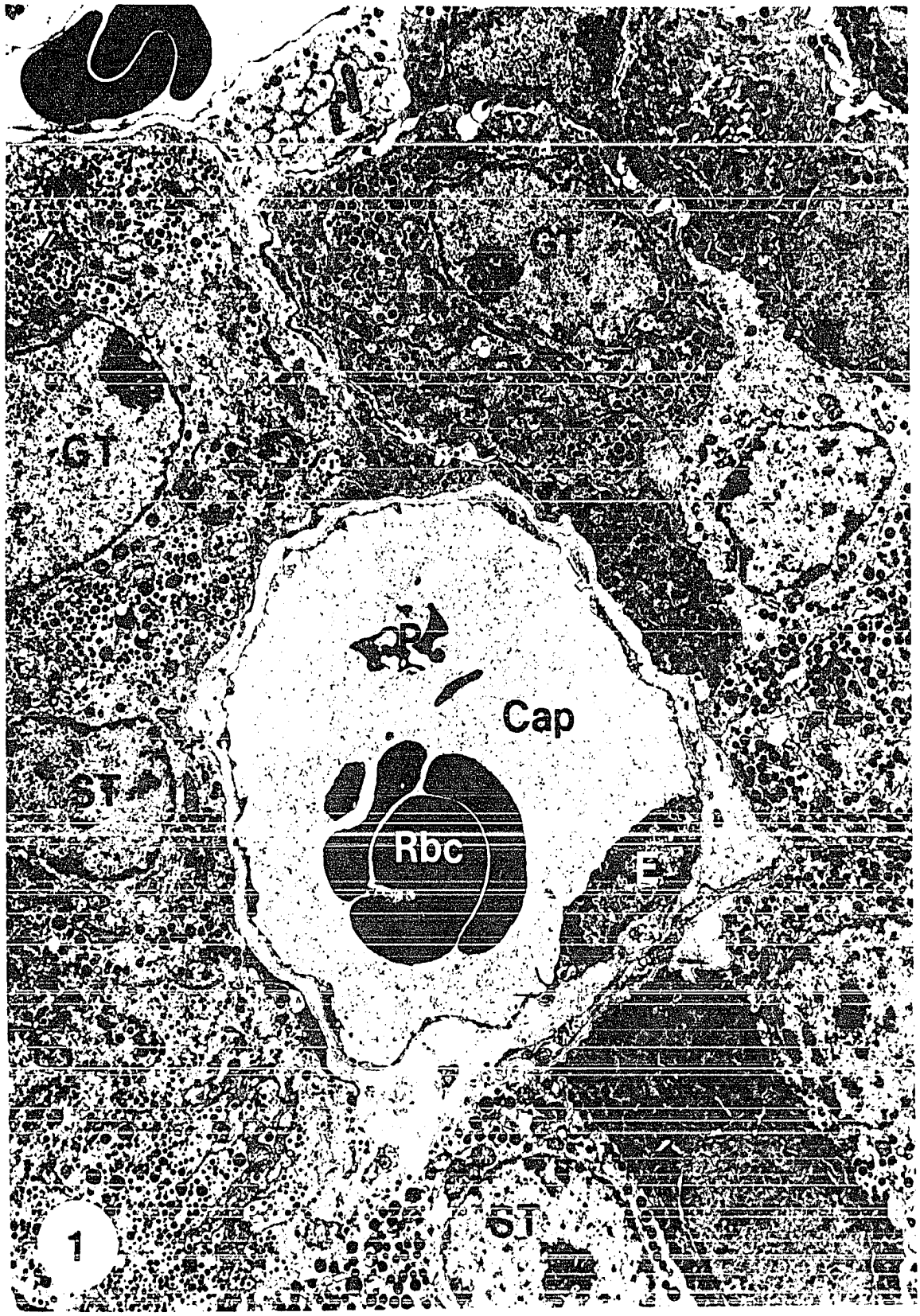
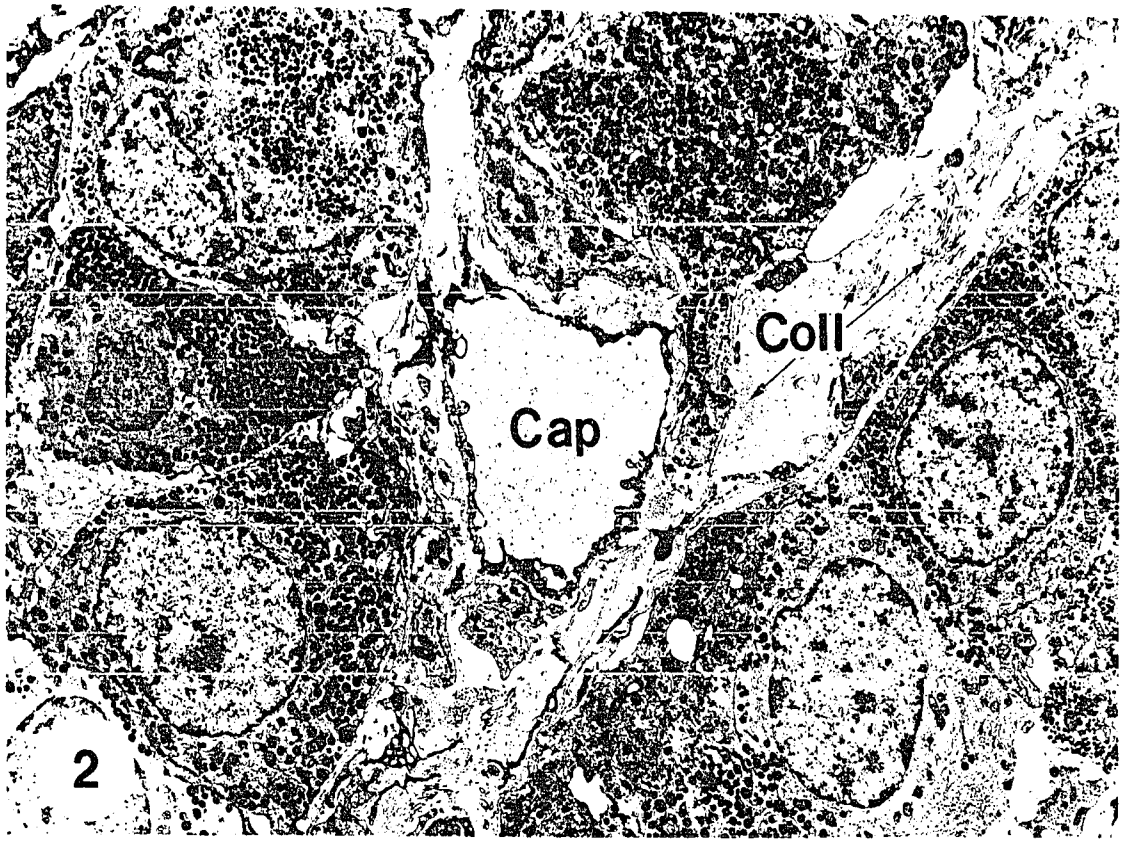

GT
GT
Cap
Rbc
1

Figure 2. Intact adenohypophysis showing capillary (Cap) and collagen (Coll). x5200.

Figure 3. Intact adenohypophysis showing: somatotroph (ST), gonadotroph (GT), follicular cells (FC), junctional complexes (jc), cilia (c) and a Golgi complex (g). x13,000.


Coll
Cap
2

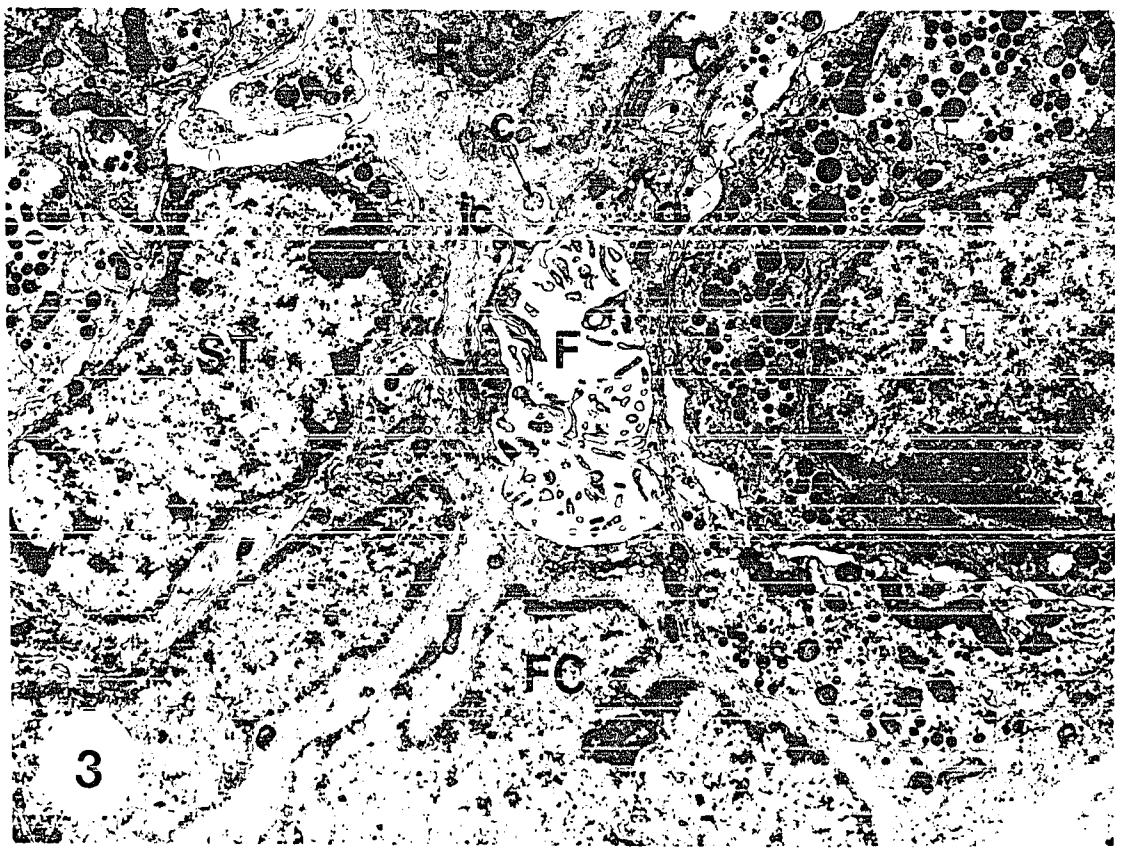
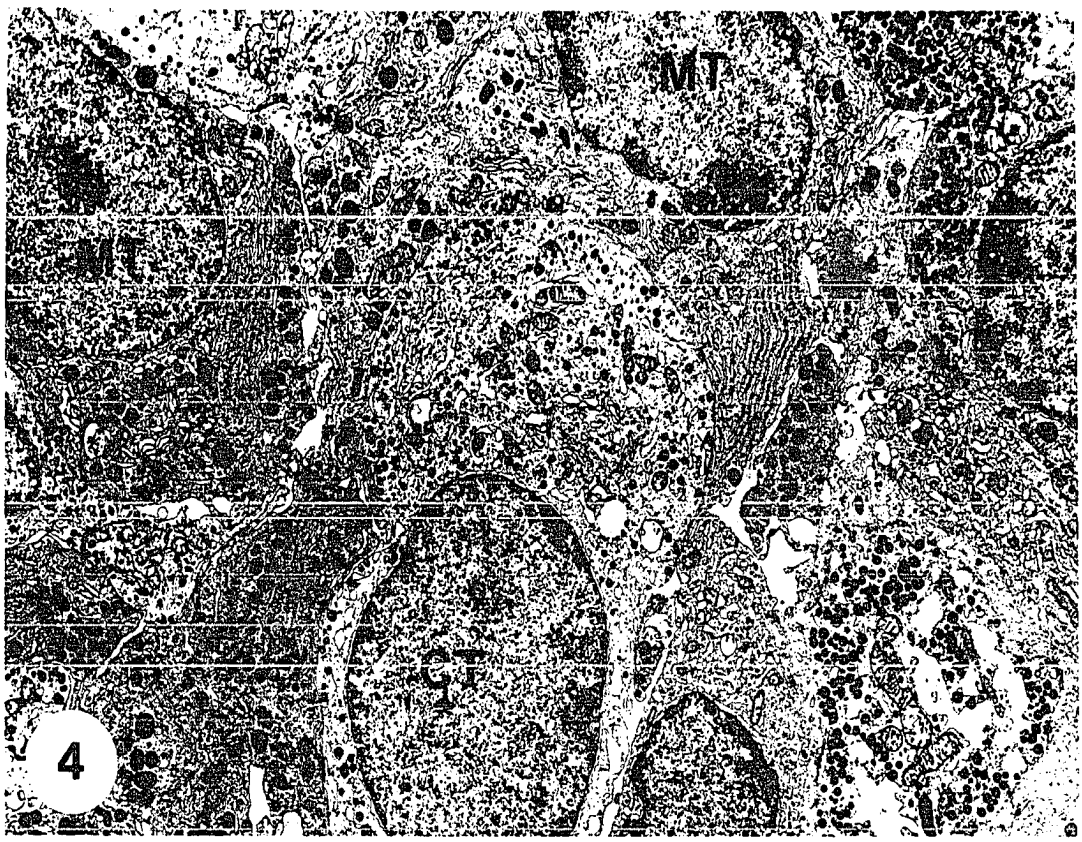

FC
FC
C
ST
F
FC
3

Figure 4. Intact adenohypophysis showing relationship between corticotrophs (CT) and mammotrophs (MT). x3500.

Figure 5. Intact adenohypophysis showing thyrotrophs (TT). x7000.


MT
MT
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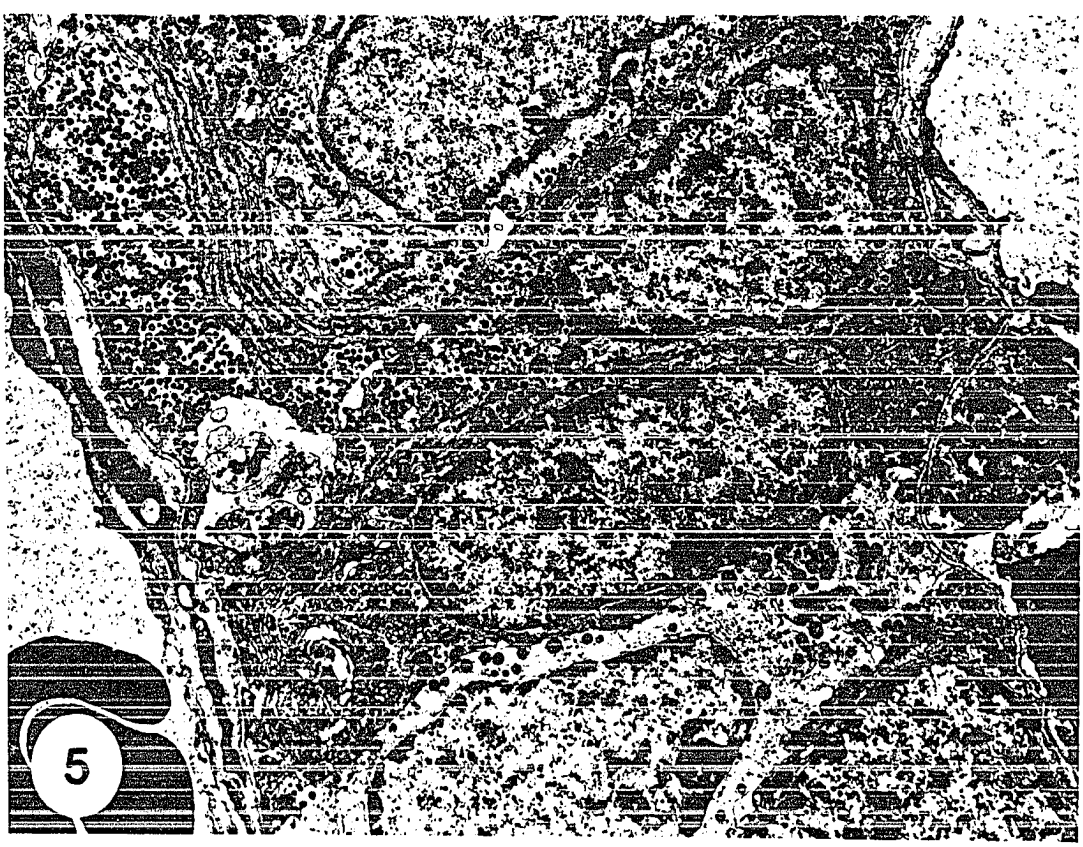
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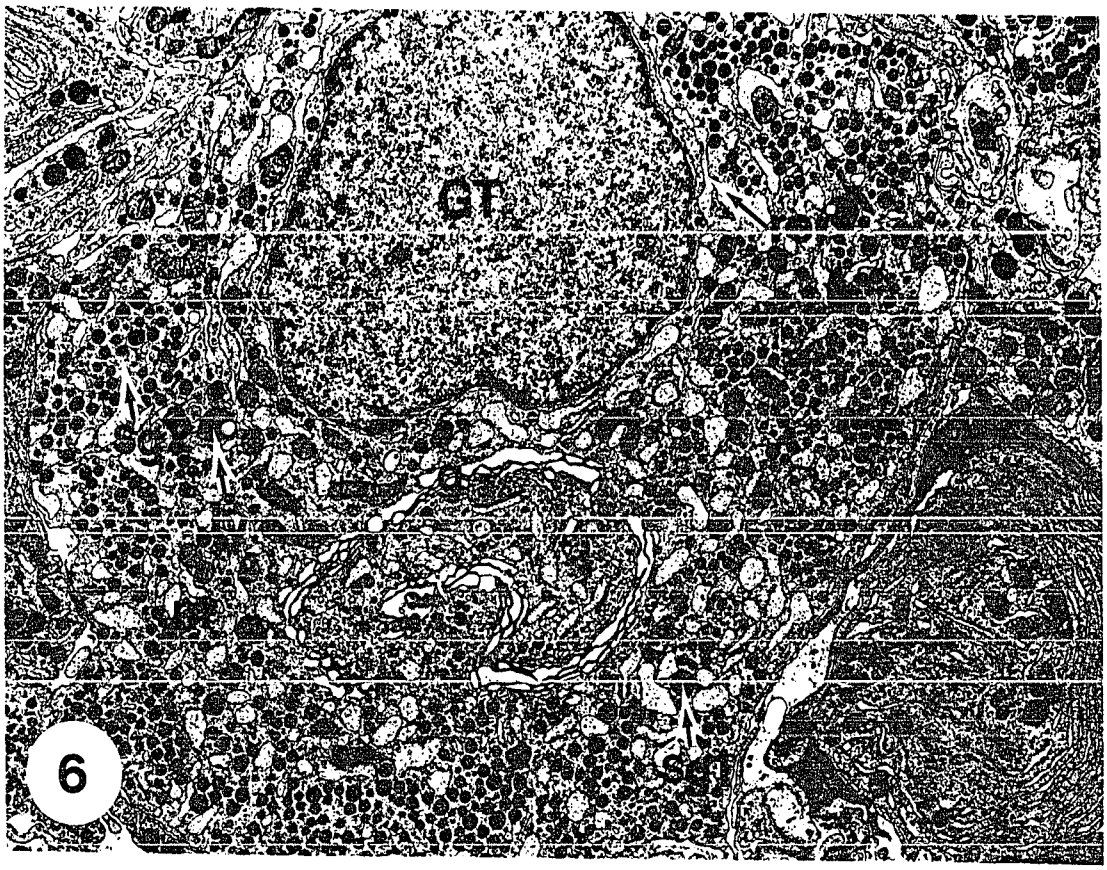
mitochondria were rod-like but a few were spherical (Figures 6 and 7). The RER was moderately developed and consisted of many slightly dilated cisternae (Figures 6 and 7). The Golgi complex was well-developed and occupied a circular area near the nucleus; immature secretory granules were observed within the dilated tips of inner cisternal elements on the trans face of the Golgi complex (Figures 6 and 7). A few lysosomes are seen in the cytoplasm (Figure 6). Coated vesicles were observed associated with portions of the smooth endoplasmic reticulum (Figure 7).

Somatotrophs Somatotrophic cells which are known to secrete growth hormone were the most frequently observed cells in the adenohypophysis (Figures 8 and 9). They were round, oval or polygonal and medium-sized (approximately 14 μ in diameter). Round, dense secretory granules about 350 nm in diameter almost filled the entire cytoplasm (Figures 8 and 9). The internal texture of these granules was homogeneous. The RER usually consisted of flat or slightly dilated cisternae, but may appear as well-developed lamellae (Figure 8). The Golgi apparatus was well-formed and consisted of many vesicles and vacuoles, but the lamellae were few. Secretory granules in various stages of elaboration were seen in some of the cisternae of the Golgi complex (Figure 9). The budding of small vesicles from the cisternae of the RER toward the cis face of the Golgi apparatus was observed (Figure 9). Coated vesicles were budding off the tubular elements in the

Figure 6. Intact adenohypophysis showing: gonadotroph (GT), rough endoplasmic reticulum (rer), Golgi complex (g), mitochondria (m), lysosome (ly), 400 nm secretory glands (sg_1) and 200 nm secretory granules (sg_2). x9000.

Figure 7. Gonadotroph in Figure 7 showing: Golgi complex (g), mitochondria (m), immature secretory granule (isg) and coated vasicle (cv). x21,600.


GT
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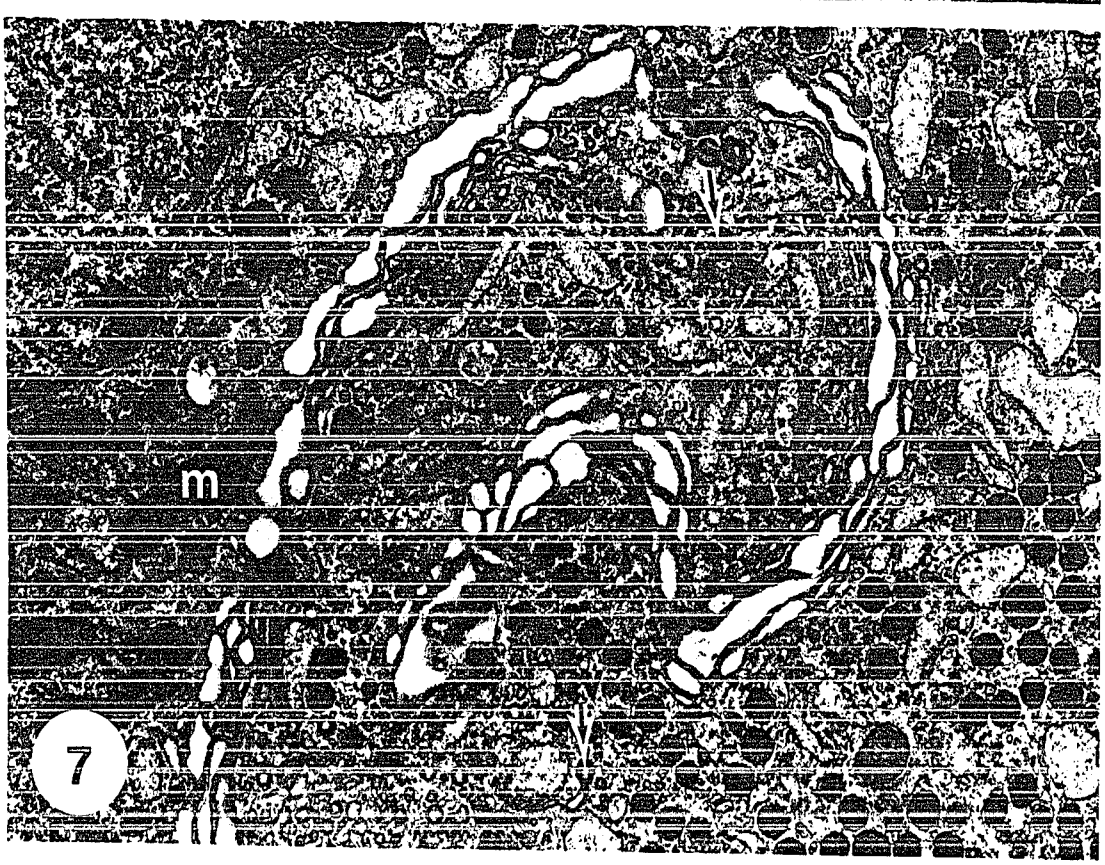
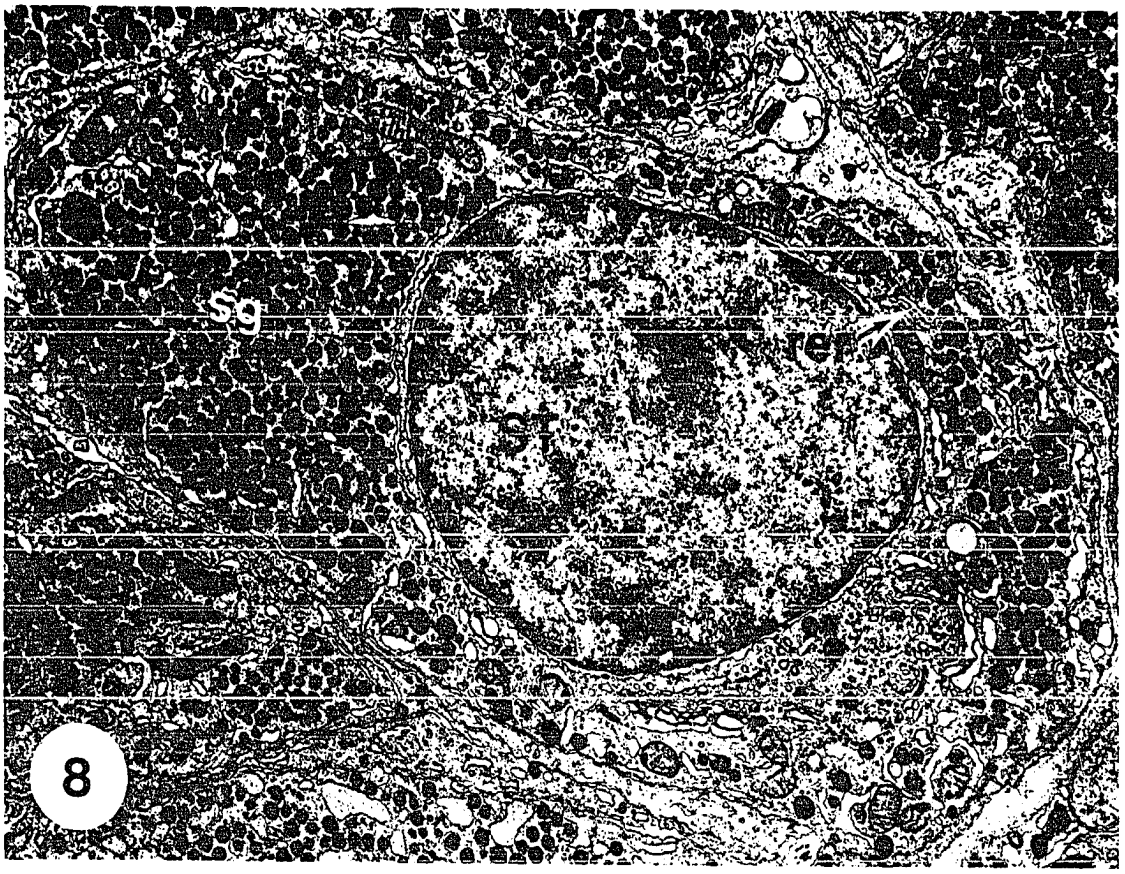

m
7

Figure 8. Intact adenohypophysis showing: somatotroph (ST), rough endoplasmic reticulum (rer), Golgi complex (g), and secretory granules (sg). x9000.

Figure 9. Somatotroph in Figure 8 showing: immature secretory granules (isg), coated vesicles (cv), small vesicles (v), and mitochondria (m). x21,600.



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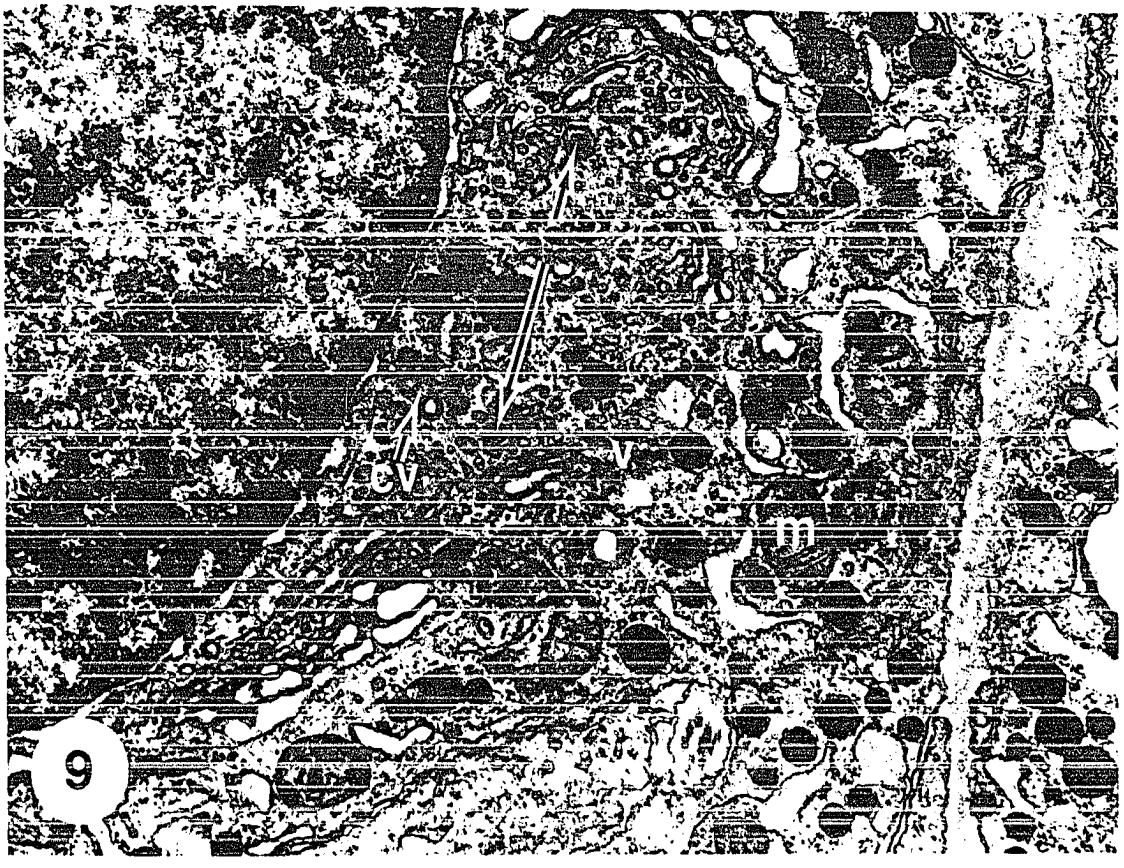

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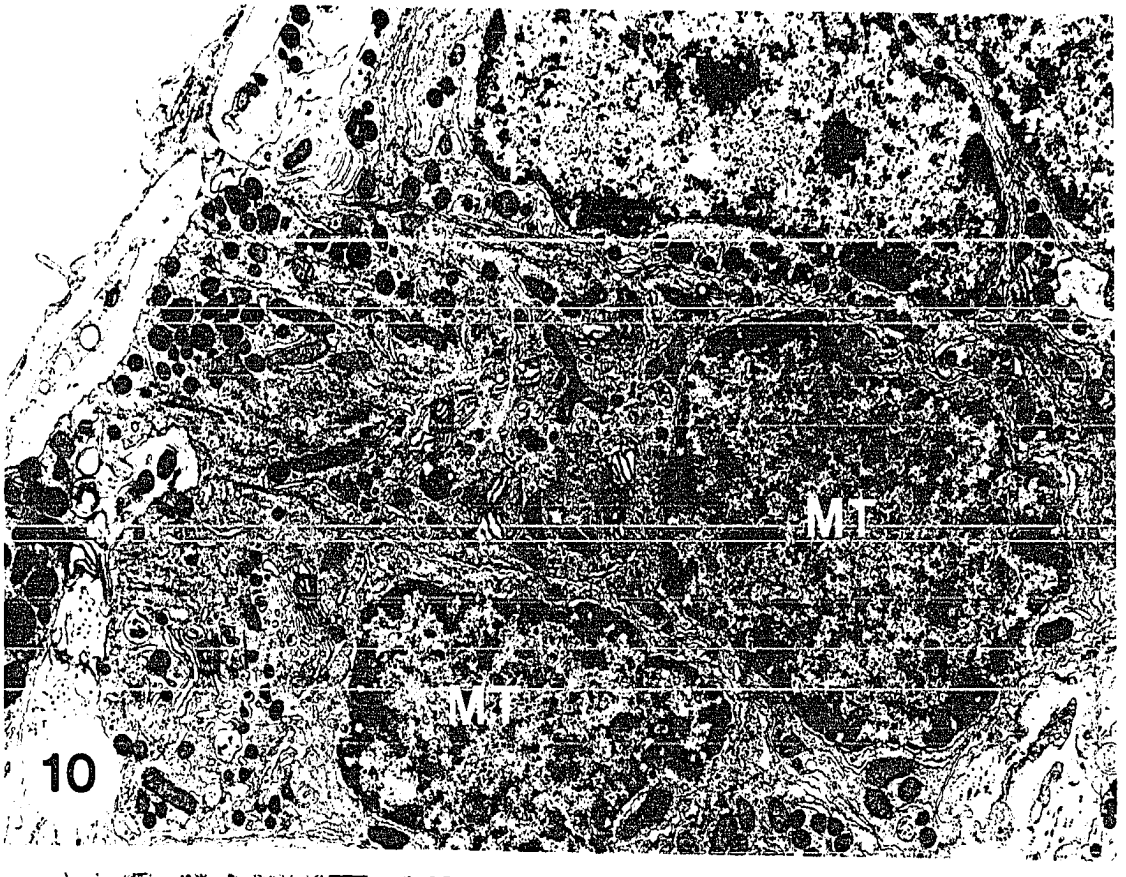
Figure 10. Intact adenohypophysis showing: mammotrophs (MT) and Golgi complexes. x9000.

Figure 11. Cytoplasm of a mammotroph in Figure 10 showing: rough endoplasmic reticulum (rer), mitochondria (m), secretory granules (sg), and Golgi complex (g). x21,600.



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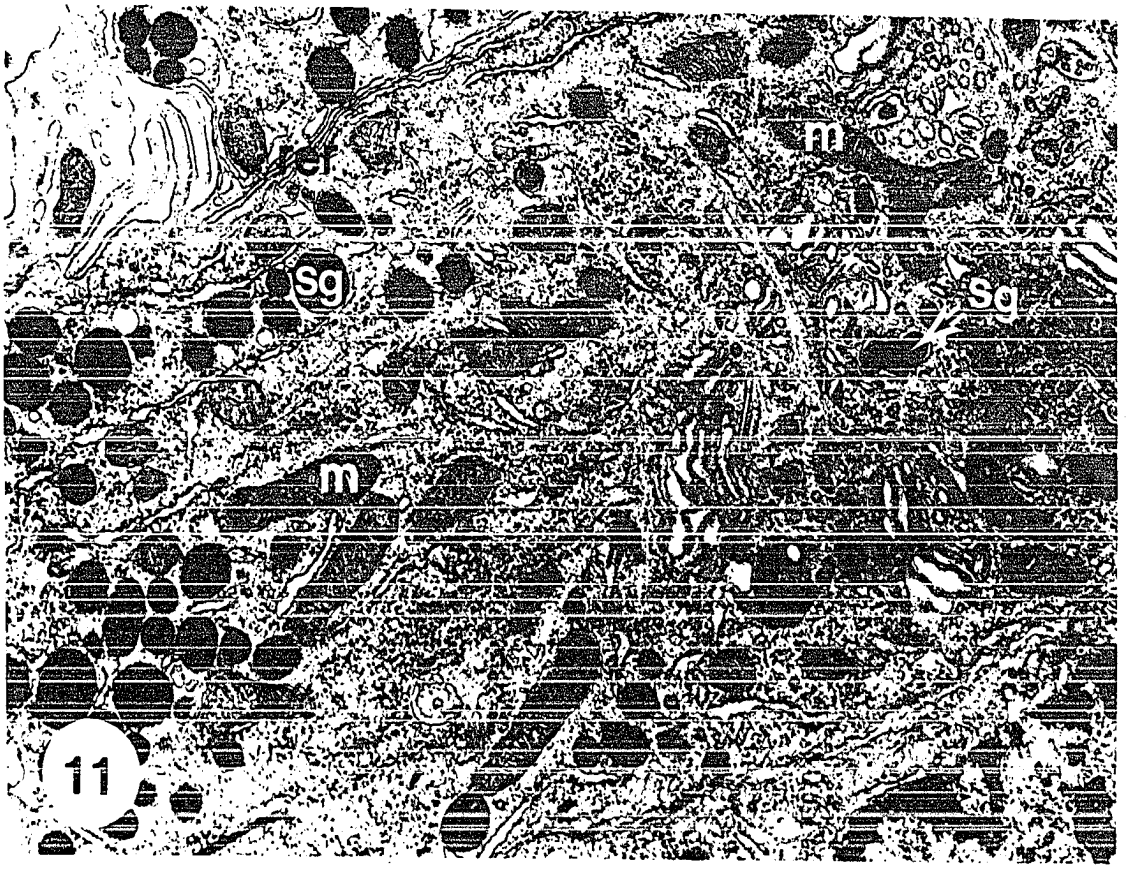


11

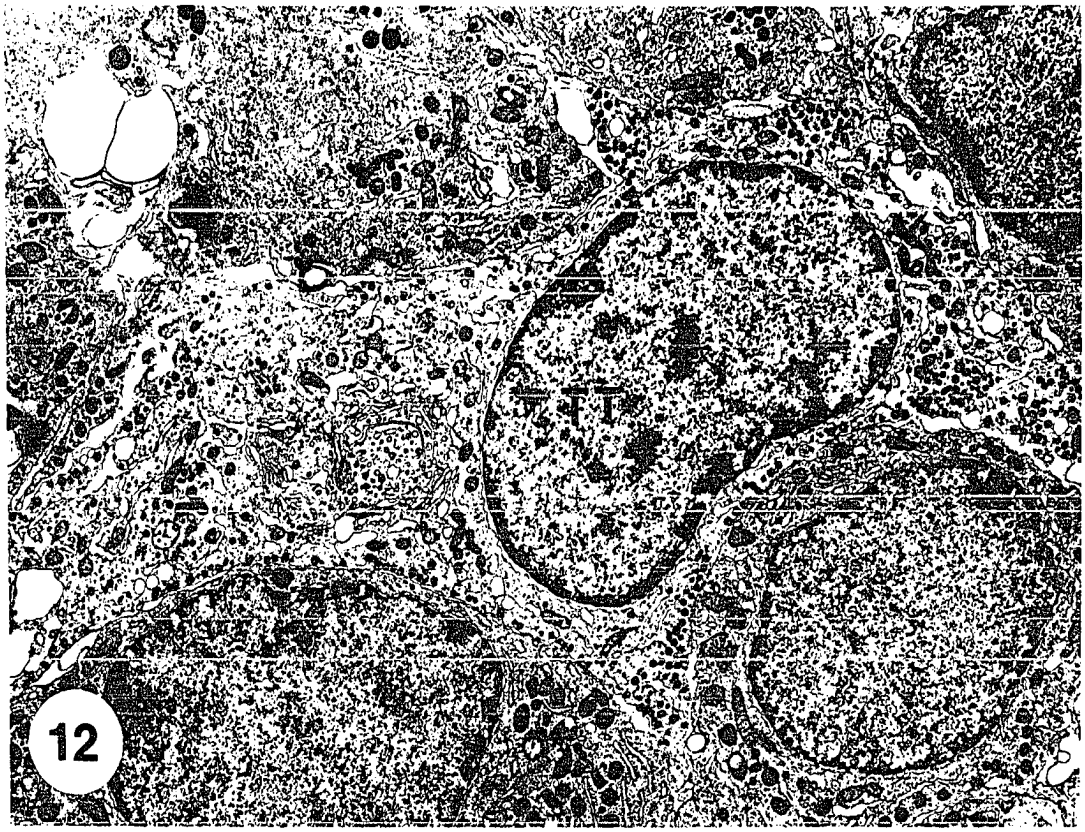
Golgi zone (Figure 9). The mitochondria were rather stout and globular-shaped (Figures 8 and 9). The mitochondrial cristae were moderate in number and intramitochondrial granules were absent.

Mammotrophs The mammotroph cells secrete prolactin. They are found abundantly in the female adenohypophysis, but sparsely in the male gland. These cells were normally about the same size as the somatotrophs (diameter of approximately 14 μ) and the cell body was round or oval in shape (Figures 4 and 10). This cell was characterized by secretory granules of irregular or elliptical shape and having a larger diameter (900 nm) than any other cell type. Generally speaking, the granules situated in the Golgi complex were smaller in size, but they were also irregular in shape (Figures 10 and 11). The RER and the Golgi apparatus of the cell were well-developed (Figure 11). The mitochondria were elongated and in one case branched (Figure 11).

Thyrotrophs Thyrotrophs are small (approximately 11 μ in diameter) cells with an angular outline (Figure 12). They contained the smallest secretory granules which measure 100-150 nm in diameter (Figure 13). Most of these granules were spherical in diameter, but some were spindle-shaped (Figure 13). They were arranged near the cell surface and were few in number. The cell organelles such as the RER, Golgi apparatus and mitochondria were poorly developed and the cytoplasm itself was also somewhat reduced (Figures 12 and 13).

Figure 12. Intact adenohypophysis showing a thyrotroph (TT). x9000.

Figure 13. Cytoplasm of the thyrotroph in Figure 12 showing: secretory granules (sg), mitochondria (m), rough endoplasmic reticulum (rer), Golgi complex (g), and coated vesicles (cv). x21,600.



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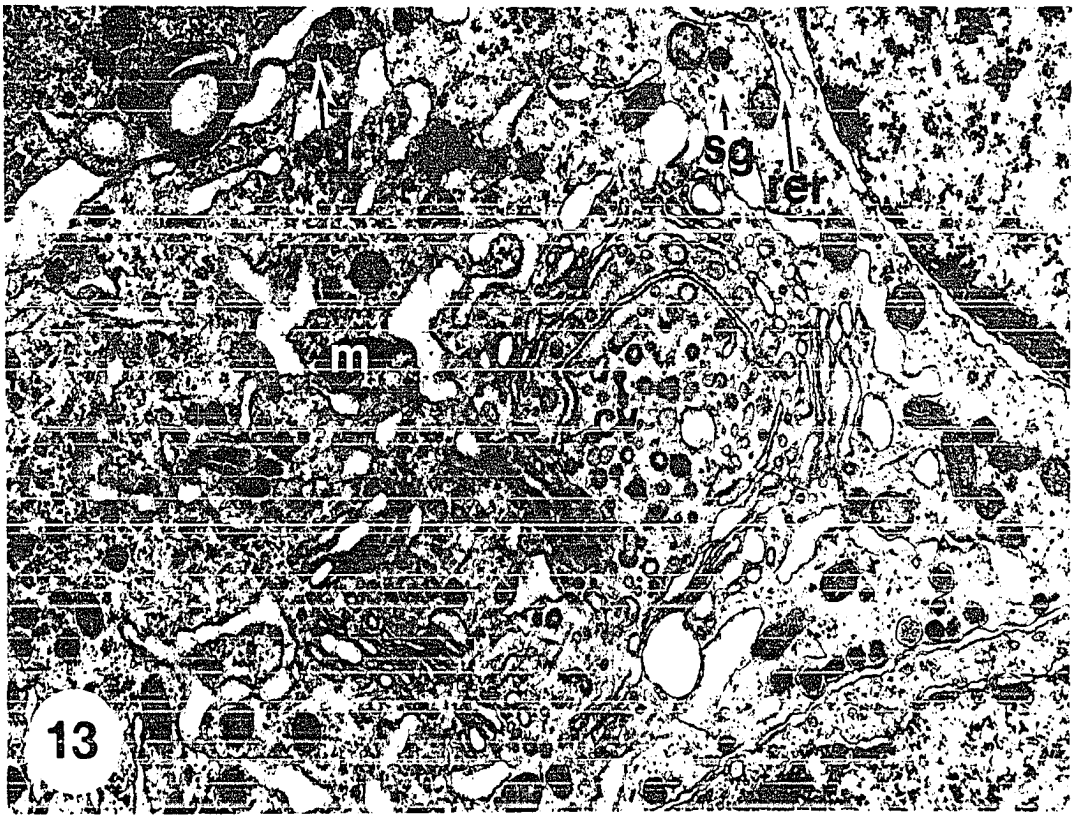


13

Coated vesicles were observed near the trans face of the Golgi apparatus (Figure 13).

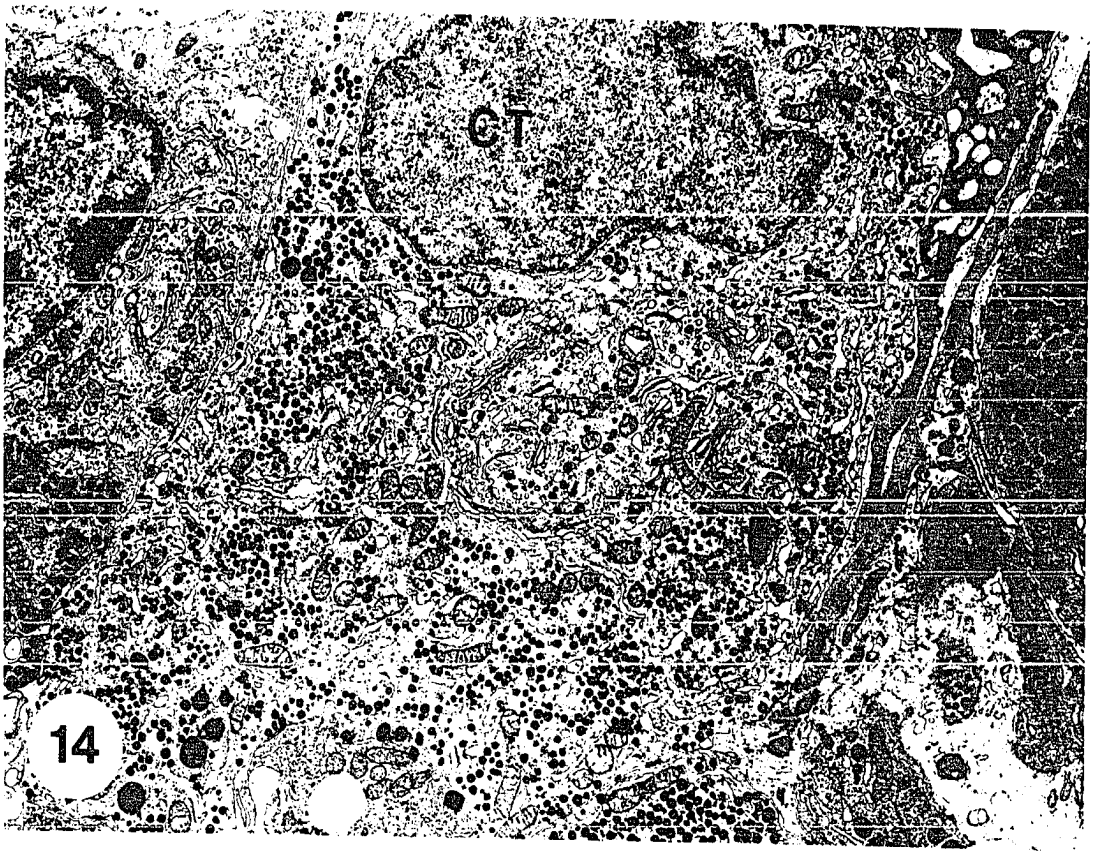
Corticotrophs Corticotrophs were large (approximately 14 μ in diameter) spherical or ovoid cells (Figures 4 and 14). They contained small secretory granules which measured 150-200 nm in diameter and were often seen as vesicles with dark cores (Figure 15). The RER was relatively simple with sparse cisternae and a small Golgi complex (Figure 15). There was a frequent association of corticotrophs with mammotrophs (Figure 4).

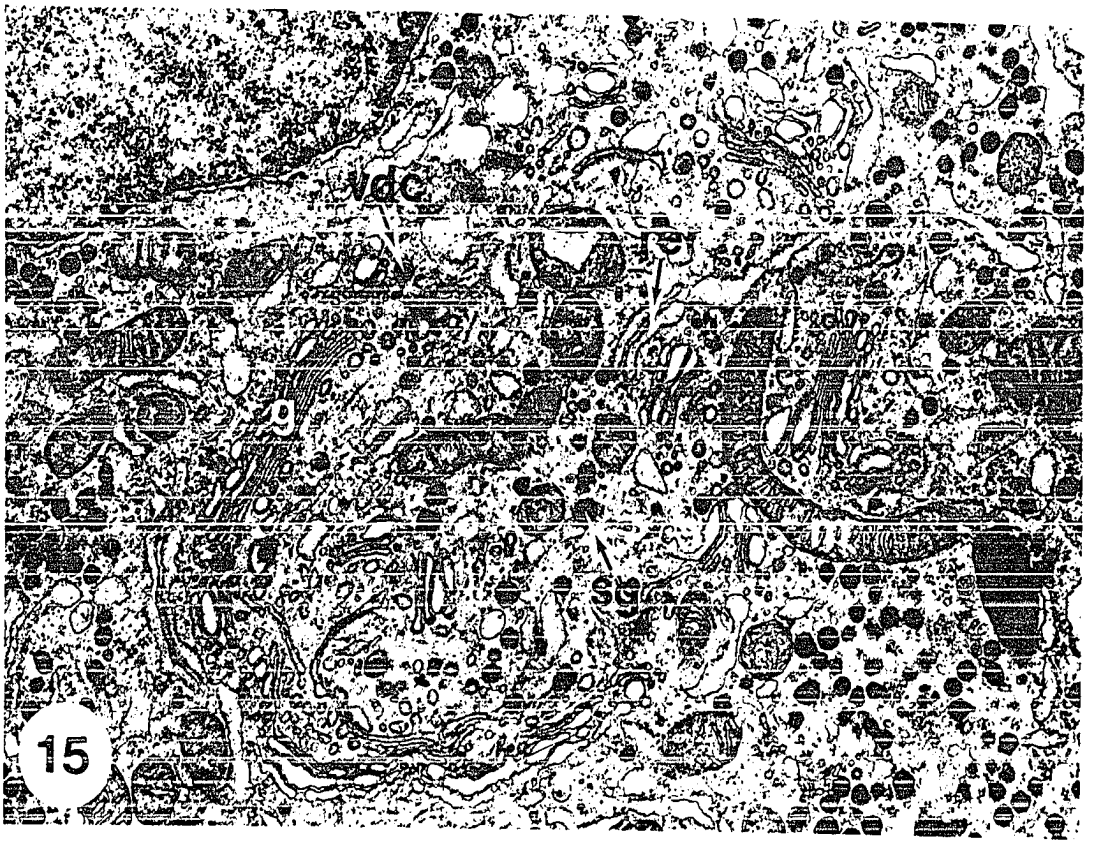
Follicular cells One type of anterior pituitary cell contained no secretory granules and this type is thought not to produce hormones but to be an undifferentiated or immature type of glandular cell. They were arranged to encircle a relatively narrow intercellular space which was either empty or filled with a colloid-like substance (Figure 3). Microvilli extended into this space from the surface of surrounding cells, and junctional complexes adjoined these nongranulated cells with each other (Figure 3). As such an arrangement of cells is reminiscent of follicles found in certain endocrine glands such as the thyroid, these cells are called follicular cells. However, the contour of such a follicle is not smooth and is not covered with the basement membrane.

The follicular cells were small in size and contained a small amount of cytoplasm with few cisternae of the RER but an abundance of free ribosomes. The Golgi complex was

Figure 14. Intact adenohypophysis showing a corticotroph (CT). x9000.

Figure 15. Cytoplasm of the corticotroph in Figure 14 showing: Golgi complex (g), rough endoplasmic reticulum (rer), secretory granules (sg), mitochondria (m), and dark cored vesicle (vdc). x18,000.


CT
14


vdc
sg
15

localized in the region between the nucleus and the cell surface facing the follicular lumen. Cilia were observed occasionally on the luminal surface (Figure 3). The follicular cells contained a few slender mitochondria. No secretory granules were found in the follicular cells.

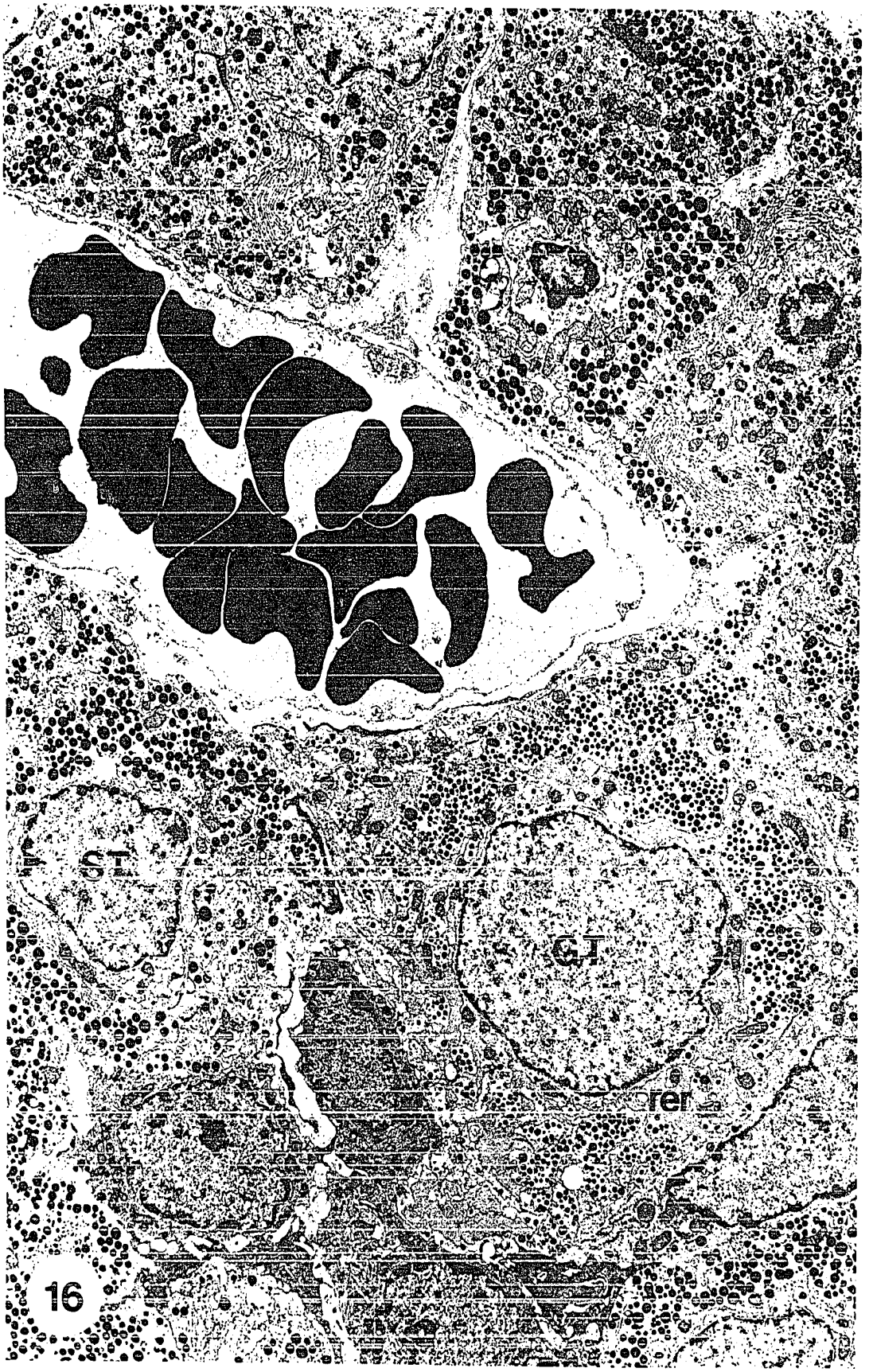
Fifteen days after castration (intact tissue)

Fifteen days following castration, the intact tissues appear unaltered with the exception of the cytoplasm of the gonadotrophic cells. The cisternae of the RER were dilated and filled with nascent, amorphous polypeptide material (Figure 16). The outer elements of the Golgi complex were more distended and dilated (Figure 18). The number of fenestrated middle elements of the Golgi complex had increased in some profiles (Figure 17). An increased number of small Golgi vesicles appeared near the trans face with some of them coalescing to form multivesicular bodies (Figures 17 and 18). Tubular and vesicular elements of the SER appeared more abundant near the trans face of the Golgi complex and immature secretory granules were observed in the inner cisternae elements of the trans face of the Golgi complex (Figure 18).

Thirty days after castration (intact tissue)

Thirty days following castration in intact tissue, the cisternae of the RER were further dilated and continued to be filled with amorphous nascent polypeptide material (Figures 19, 20 and 21). The outer elements of the Golgi complex had

Figure 16. Intact adenohypophysis 15 days after castration showing: somatotroph (ST), gonadotroph (GT), and rough endoplasmic reticulum (rer). x4500.


GT
GT
rer
16

distended further and become more dilated (Figure 20). Some of the secretory granules had a dense core (Figure 21).

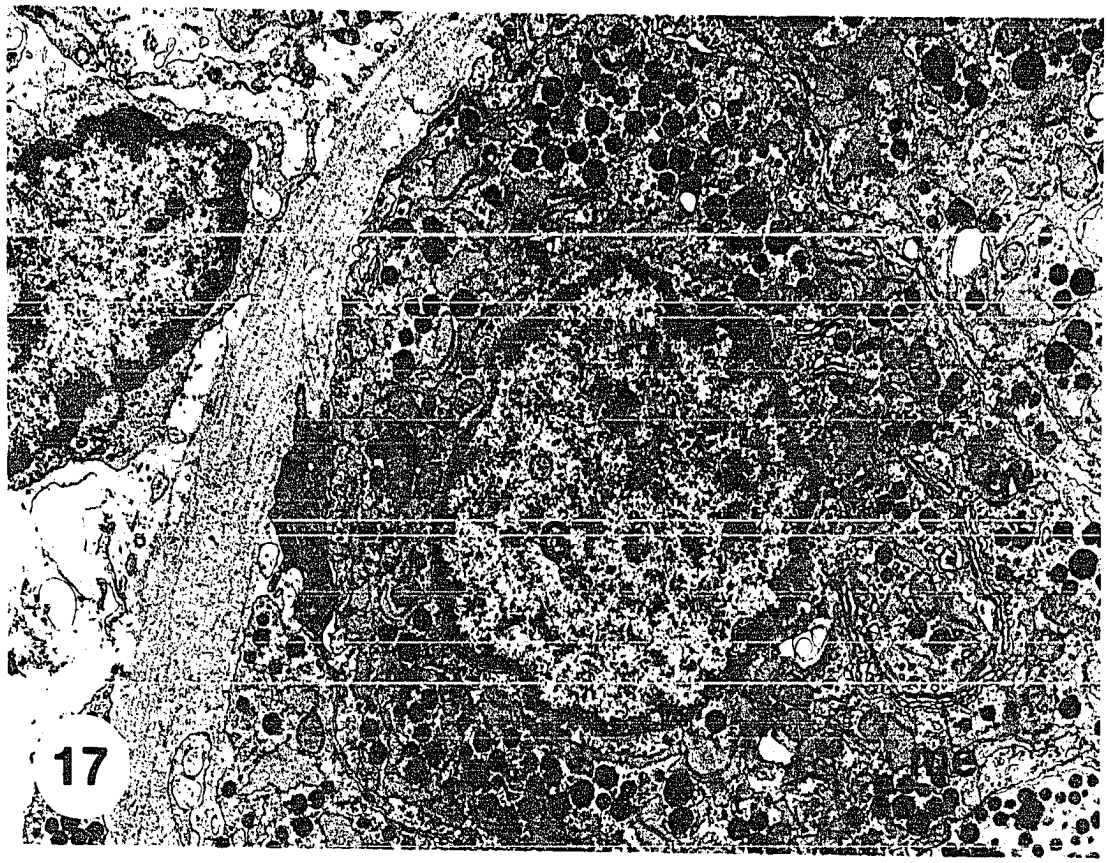
Normal cells (dissociated tissue)

Following dissociation, the various cell types of the anterior pituitary appeared to be well-preserved and functional cells with some exaggerated cytoplasmic vacuolization (Figures 22, 24, 26, 28, 30, 32 and 33) were present.

Electron microscopic observations indicate that all of the cell types observed in intact tissue were represented; there was no indication of any cell type being preferentially lost. At least five secretory cell types--gonadotrophs (Figures 22 and 23), somatotrophs (Figures 24 and 25), mammotrophs (Figures 26 and 27), thyrotrophs (Figures 28 and 29), and corticotrophs (Figures 30 and 31)--were recognized based on their previously described morphologic features. In addition, the nonsecretory cell types, follicular cells (Figure 32) and endothelial cells (Figure 33), were present. Figures 22-33 illustrate the fine structural organization of the cells as they appeared immediately after dissociation. The cells lost their varied shapes and became rounded up. In the process, some redistribution of their intracellular components took place--most notably, the Golgi apparatus assumed a more predominantly juxtanuclear position (Figures 23, 25, 27, 29 and 31). With the exception of the Golgi complex, there was no obvious change in the distribution of the subcellular

Figure 17. Intact adenohypophysis 15 days after castration showing: gonadotroph (GT), Golgi vesicles (gv), and middle cisternal elements of the Golgi complex (me). x9000.

Figure 18. Cytoplasm of the gonadotroph in Figure 17 showing: immature secretory granule (isg), outer elements of the Golgi complex (oe), vesicular (v), and tubular (t) elements of the Golgi complex. x18,000.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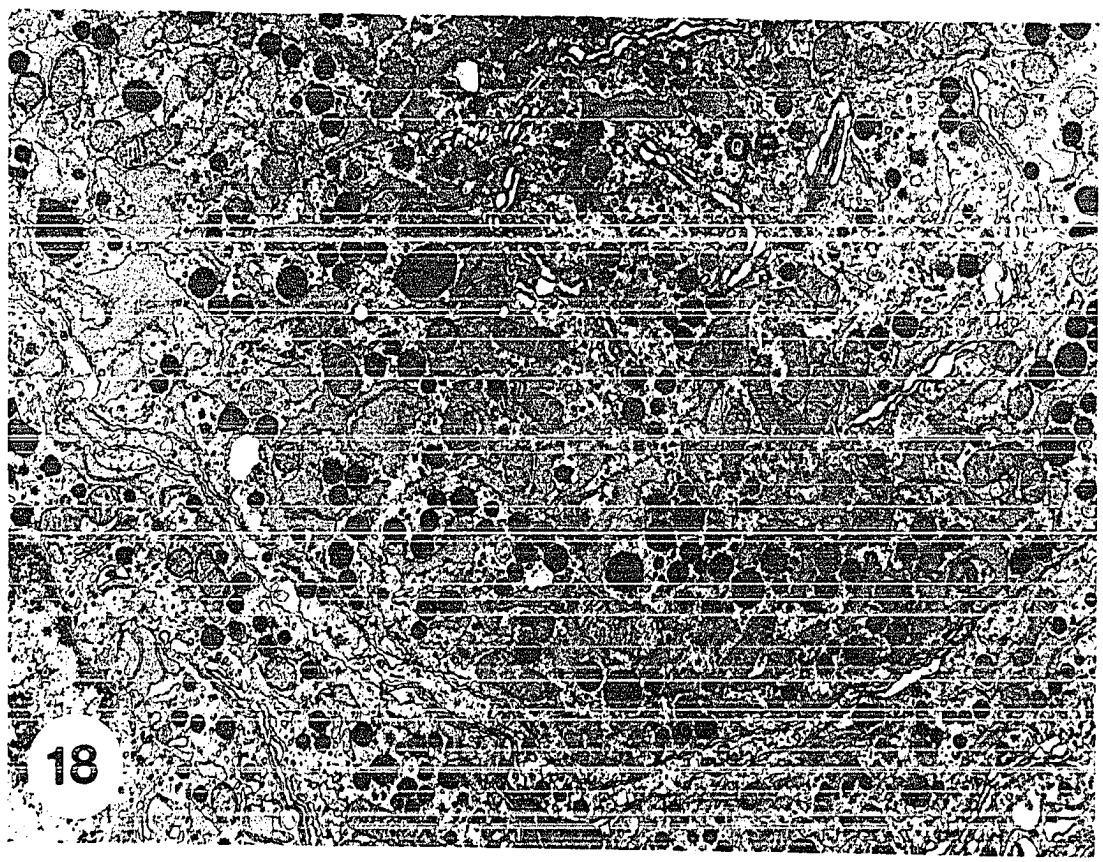
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Figure 19. Intact adenohypophysis 30 days after castration. x18,000.

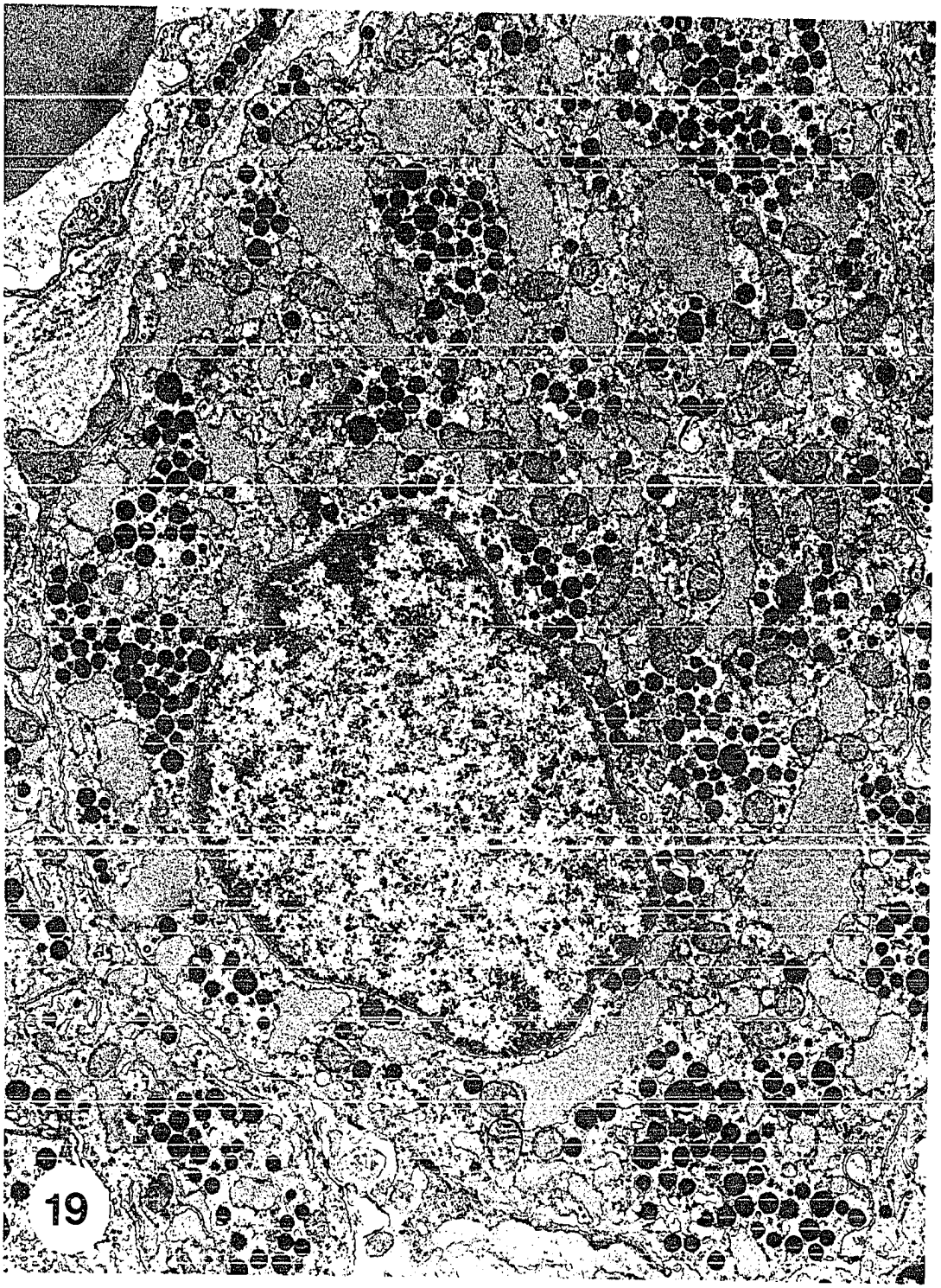
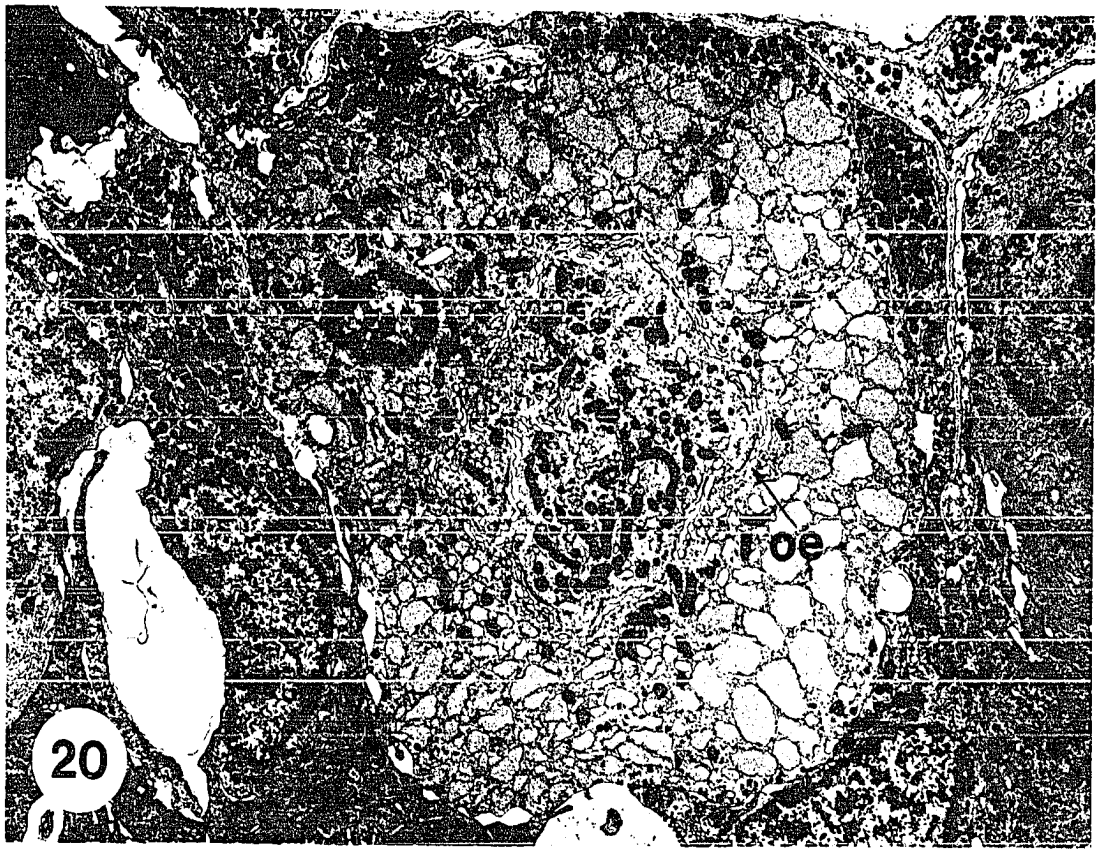
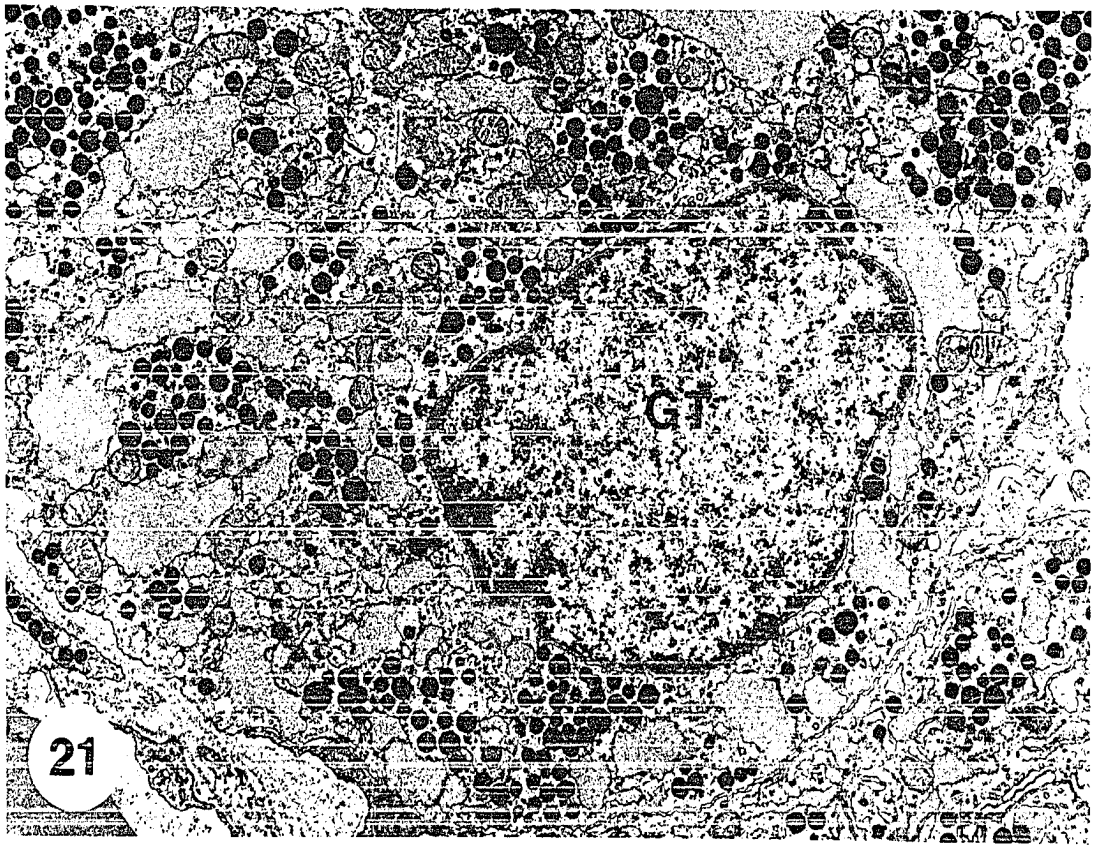

19

Figure 20. Intact adenohypophysis 30 days after castration showing a gonadotroph and outer elements (oe) of the Golgi complex. x9000.

Figure 21. Intact adenohypophysis 30 days after castration showing a gonadotroph (GT) and secretory granules (sg). x18,000.


oe
20


GT
21

organelles.

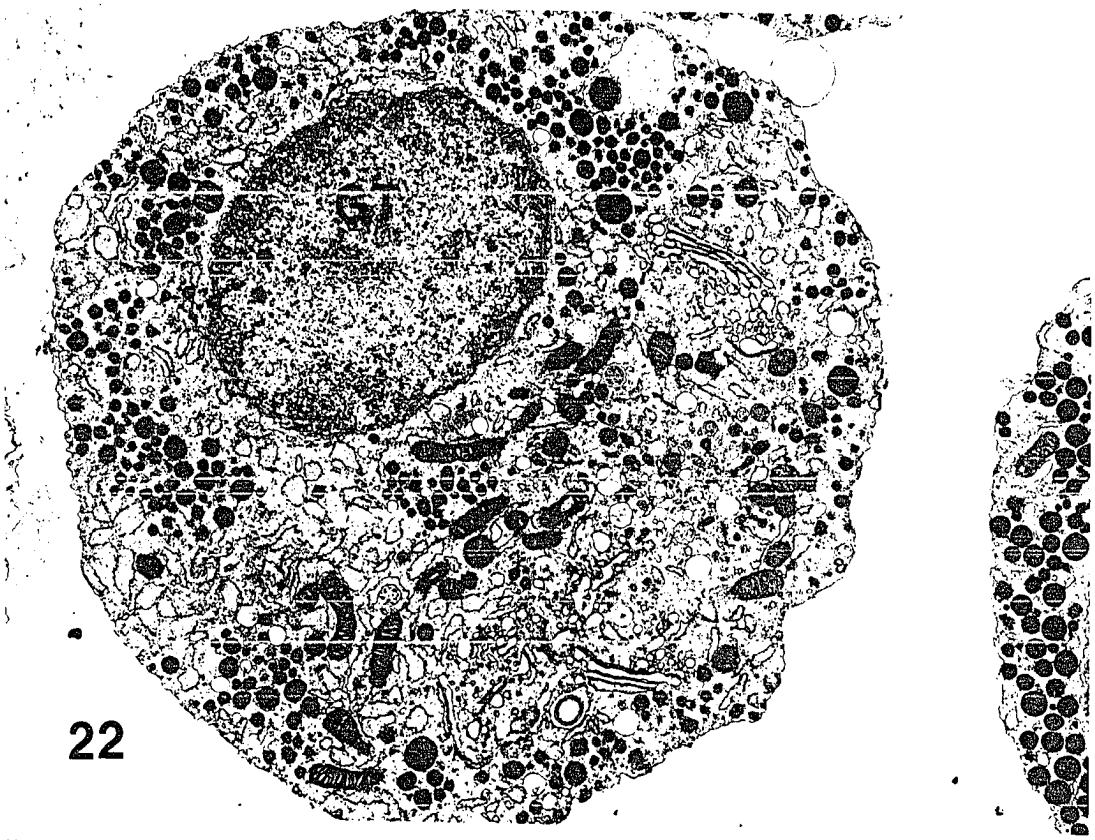
Following dissociation, the normal gonadotrophs appeared to be well-preserved functional cells with some vacuolization in the cytoplasm (Figures 22 and 23). These cells contained both large (400 nm) and small (200 nm) secretory granules and the cisternae of the RER appeared slightly distended (Figures 22 and 23). The mitochondria appeared as they do in the cells from intact tissue as did the nucleus (Figures 22 and 23). The Golgi complex appeared to have the normal number and arrangement of outer, middle and inner cisternal elements (Figure 23). There were numerous small Golgi vesicles near the trans face of the Golgi complex with some tubular and cisternal elements of the SER adjacent to the trans face of the Golgi complex (Figure 23).

Fifteen days after castration (dissociated tissue)

Dissociated cells fifteen days after castration had dilated cisternae of the RER and vacuolization in the cytoplasm (Figures 34 and 35). The outer, middle fenestrated and inner cisternal elements of the Golgi complex were hypertrophied and there were immature secretory granules seen forming on the trans face of the Golgi complex (Figures 35). Both sizes of secretory granules were observed and appeared to have been pushed to the periphery of the cell as was the nucleus by the distended Golgi complex and RER (Figure 34). There were also SER components and large numbers of small

Figure 22. Dissociated adenohypophysis showing a gonadotroph (GT). x8000.

Figure 23. Cytoplasm of gonadotroph in Figure 22 showing mitochondria (m), rough endoplasmic reticulum (rer), vacuoles (vac), 400 nm secretory granules (sg1), 200 nm secretory granules (sg2), transitional (t) and vesicular (v) elements of the Golgi complex (g). x16,000.


G
22

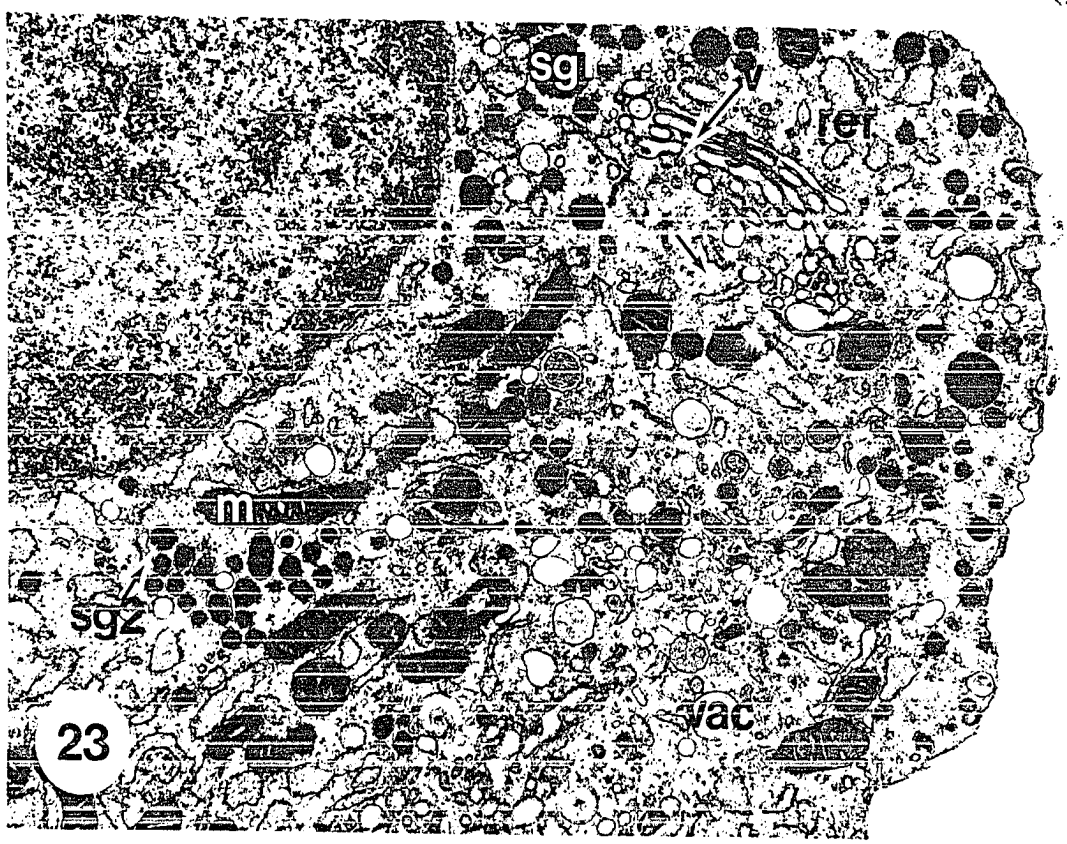
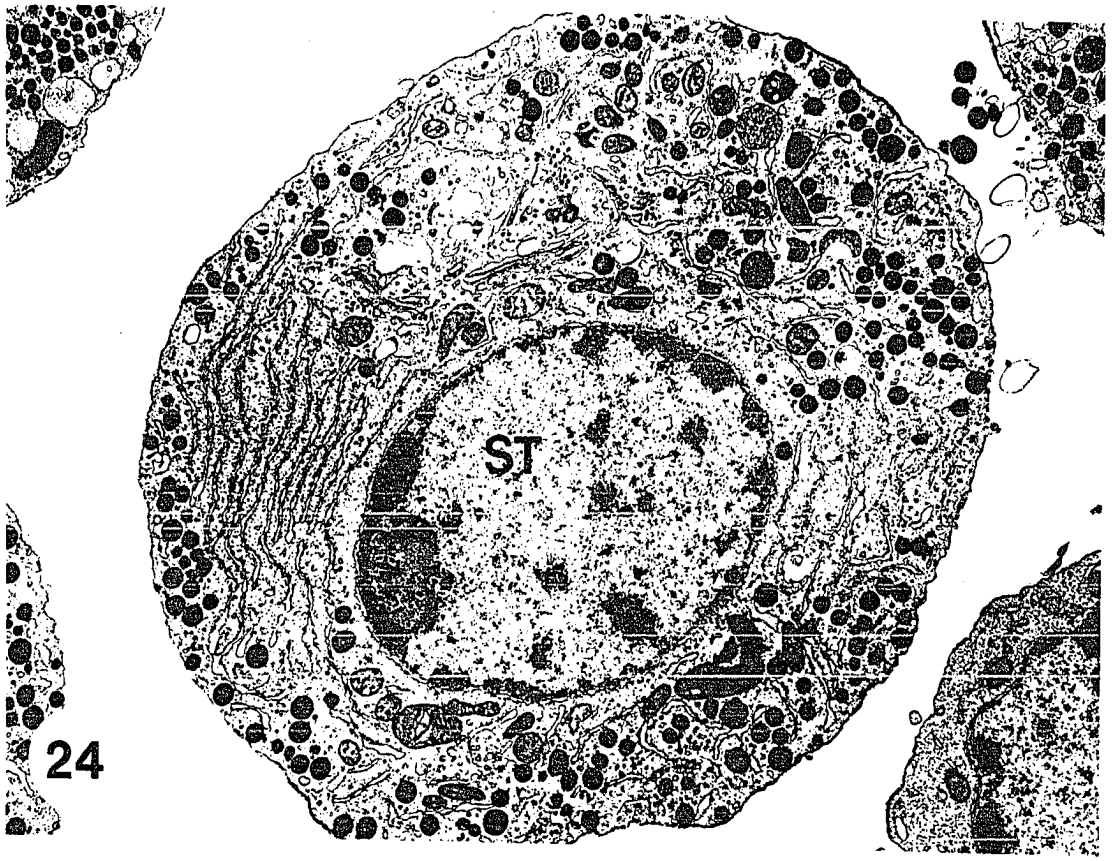

sg1
v
ret
m
sg2
vac
23

Figure 24. Dissociated adenohypophysis showing a somatotroph (ST). x9000.

Figure 25. Cytoplasm of the somatotroph in Figure 24. x18,000.


ST
24

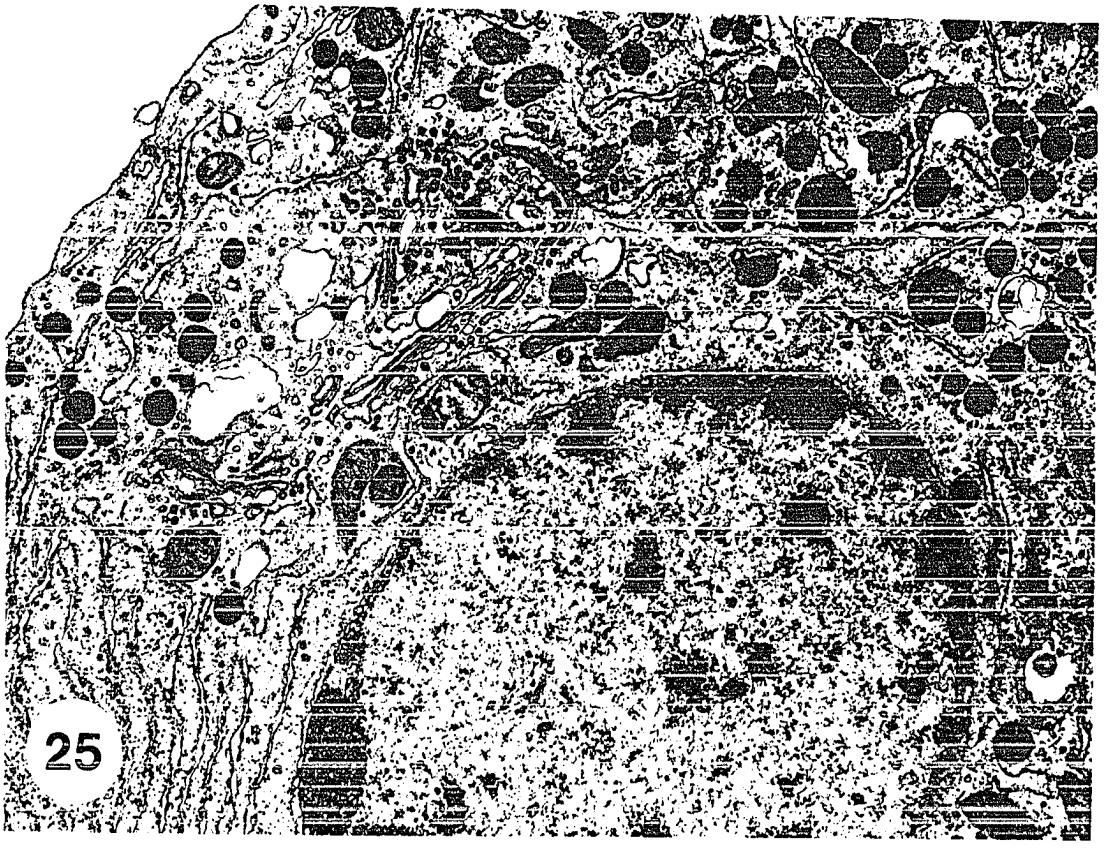

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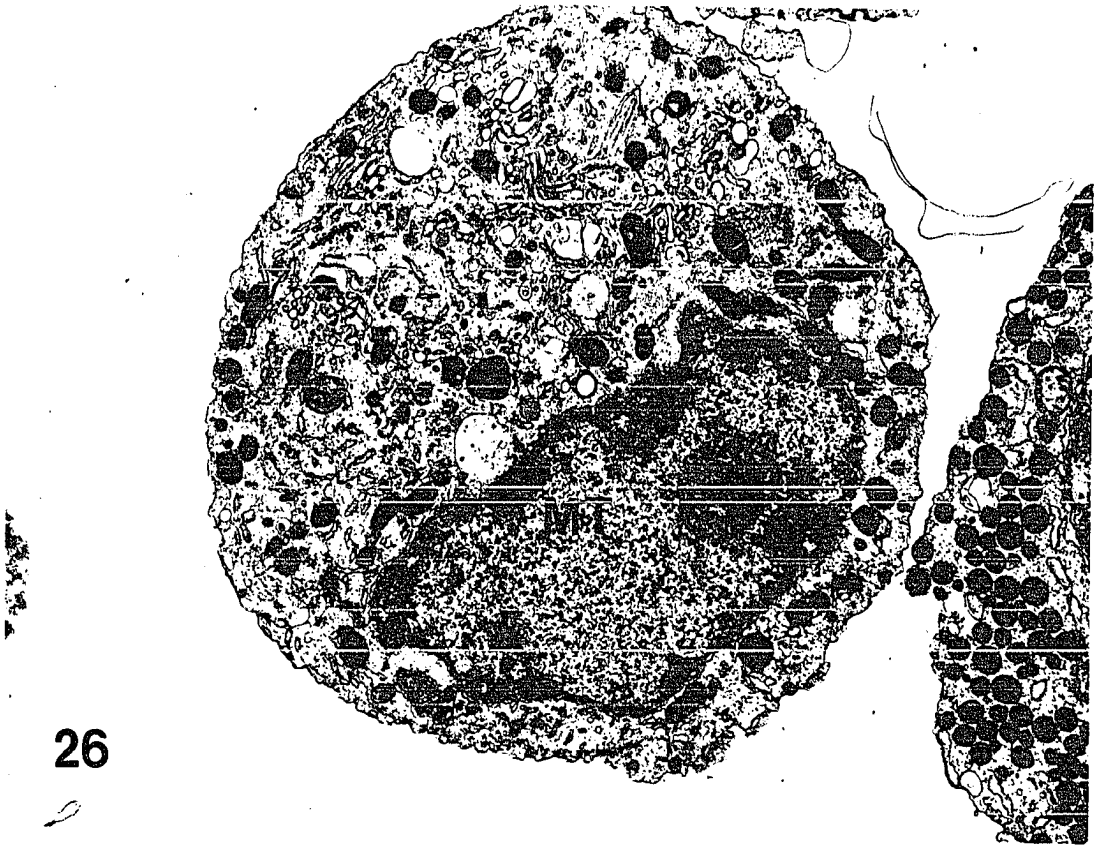
Figure 26. Dissociated adenohypophysis showing a mammotroph (MT). x9000.

Figure 27. Cytoplasm of the mammotroph in Figure 26. x18,000.



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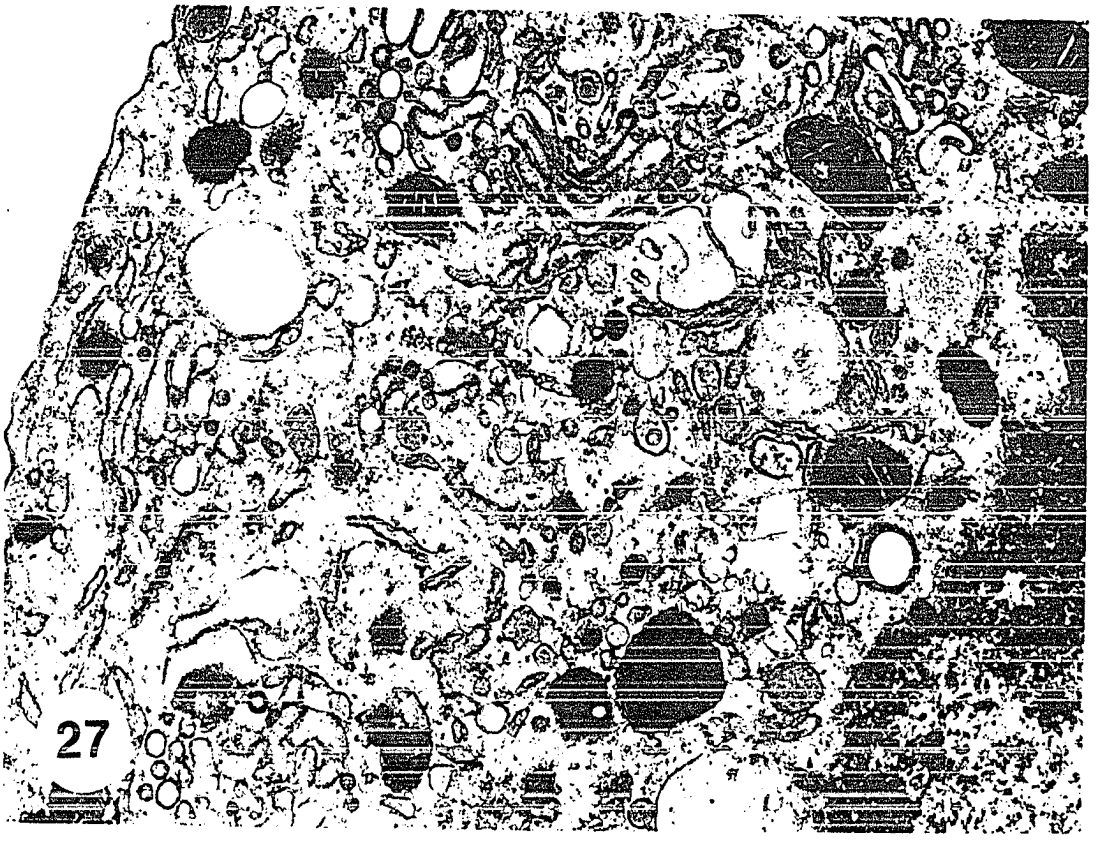


27

Golgi vesicles near the cis and trans face of the Golgi complex (Figure 35).

Thirty days after castration (dissociated tissue)

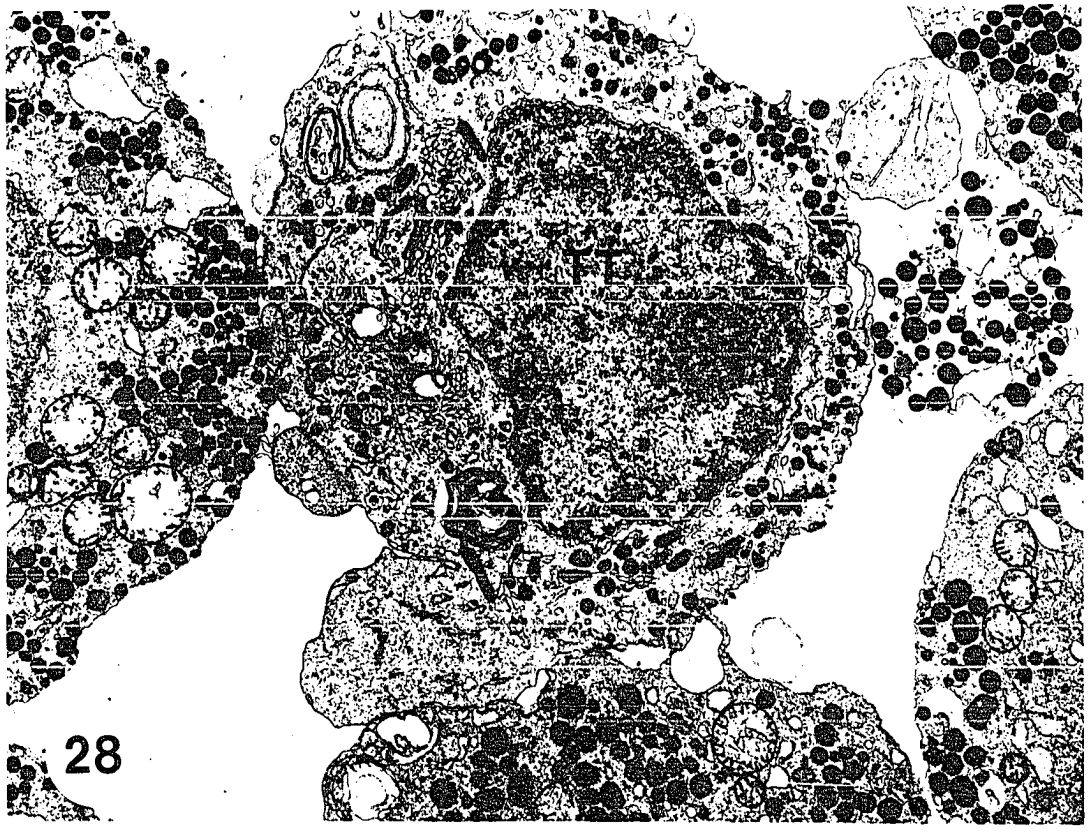
Dissociated gonadotrophs thirty days after castration had a RER with enormous cisternae (Figure 36). There were both types of secretory granules which had been pushed to one side of the cell (Figure 36). The Golgi complex had increased numbers of cisternae. Immature secretory granules were observed in cisternal elements on the trans face of the Golgi complex (Figure 37). The mitochondria and the nucleus appeared well preserved (Figures 36 and 37).

Nonsecretory cells (dissociated tissue)

There are two principal nonsecretory elements present in the anterior pituitary, the follicular and the endothelial cells (Figures 32 and 33). Where secretory cells were rounded and had relatively smooth contours after dissociation, these cell types had slightly irregular and elaborate contours with numerous microvillous processes (Figures 32 and 33). The endothelial cells line the capillaries of the pituitary and they have long convoluted fenestrae which distinguish them from the follicular cells. The follicular cells are unique pituitary components which *in situ* are arranged in groups forming the lining of small follicles of ductules and are attached to one another along their luminal surfaces by apical junctional complexes. After dissociation follicular

Figure 28. Dissociated adenohypophysis showing a thyrotroph (TT). x9000.

Figure 29. Cytoplasm of the thyrotroph in Figure 28. x18,000.



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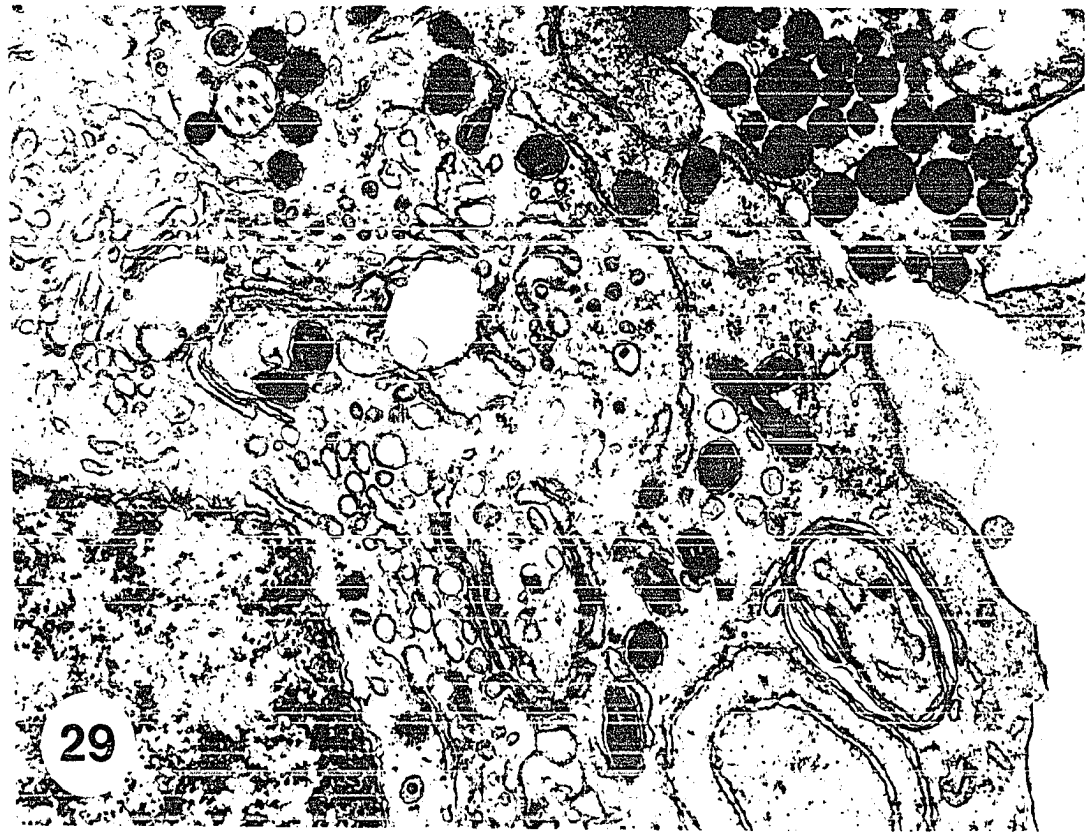

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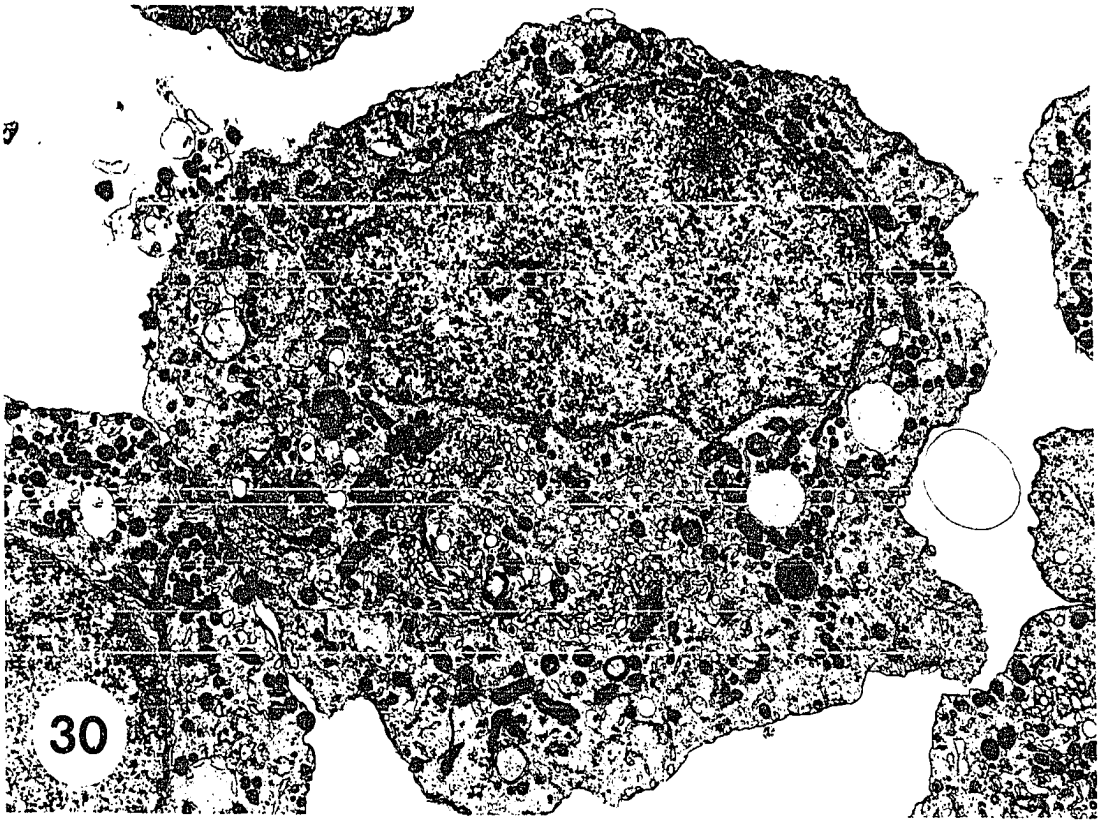
Figure 30. Dissociated adenohypophysis showing a corticotroph (CT). x9000.

Figure 31. Cytoplasm of the corticotroph in Figure 30. x18,000.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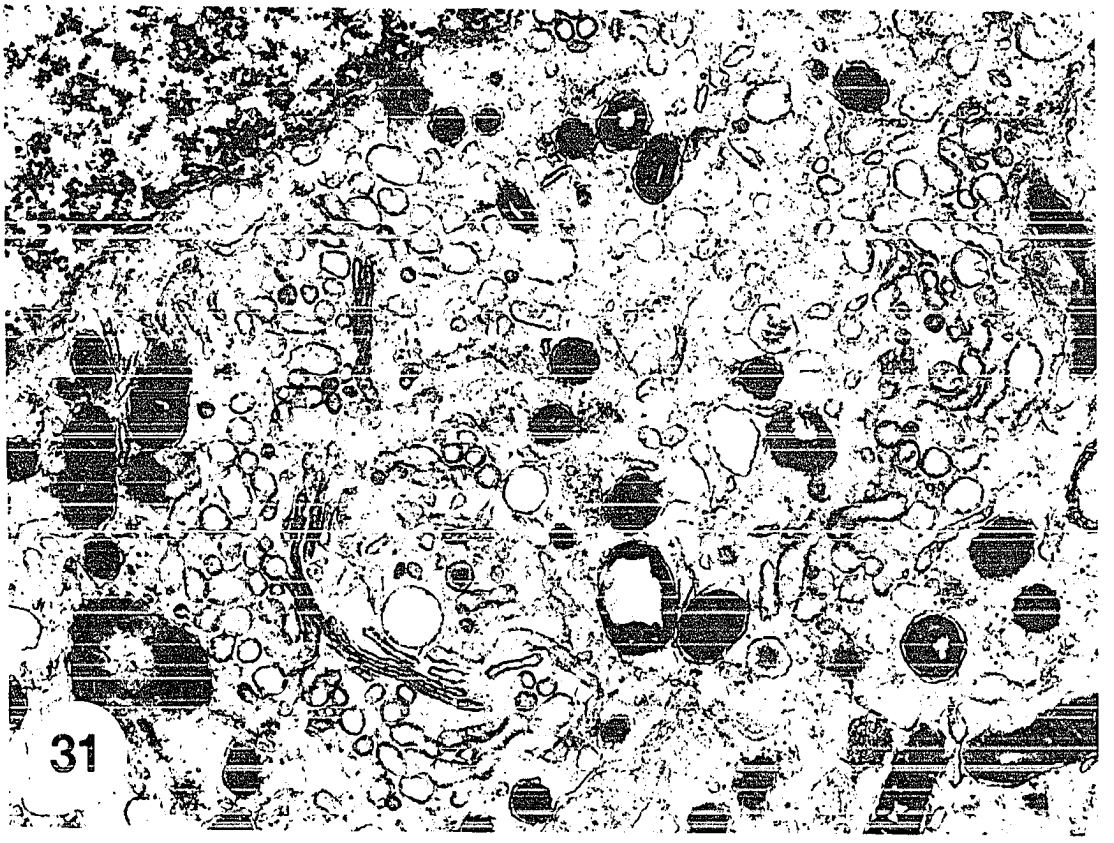

31

cells were easily distinguished from endothelial cells by their lack of long convoluted fenestrae and the presence of thicker, shorter microvilli (Figures 32 and 33).

Study of Gonadotropin Synthesis

The ability of gonadotroph cells to synthesize, transport and release secretory products was analyzed by employing labeled synthetic precursors dissolved in the medium surrounding the dissociated cells in a pulse-chase experimental protocol with autoradiographic localization of the label. Two labels, leucine-^{3}H and mannose-^{3}H were utilized. The cells were stimulated to produce synthetic product by removing the negative feedback from the testis through castration.

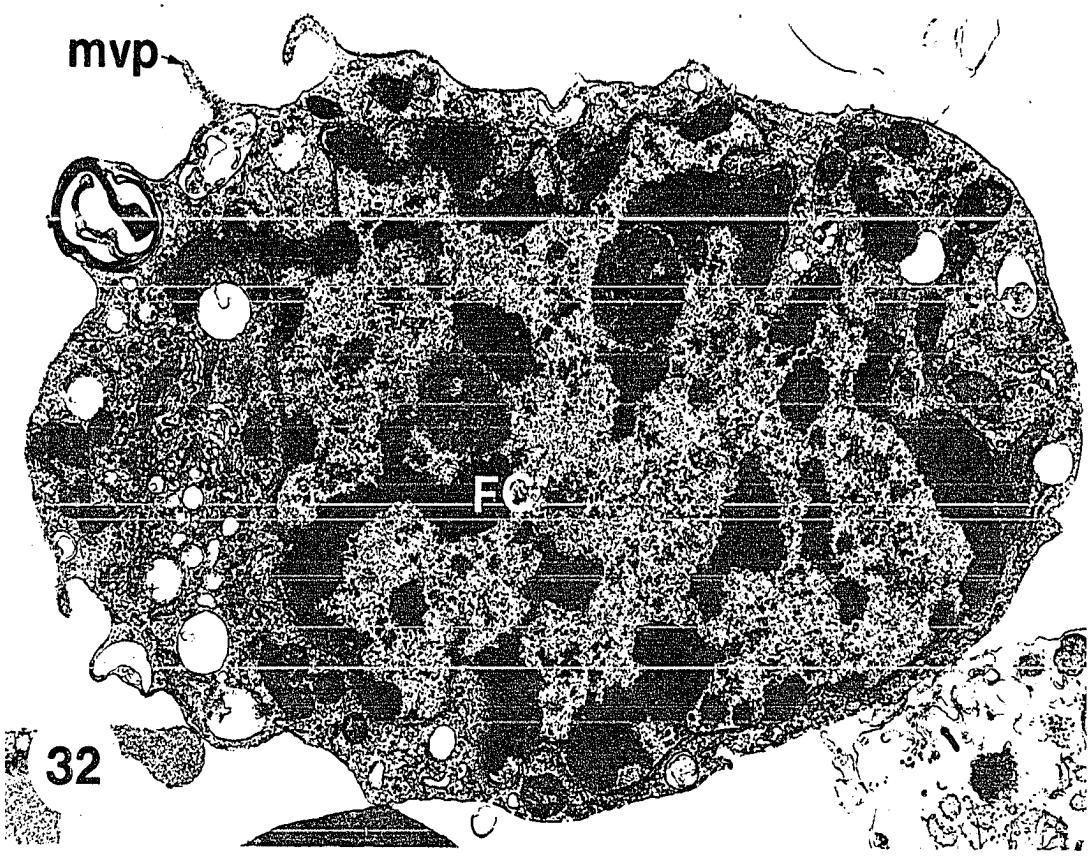
Pulse labeling with leucine-^{3}H

The distribution of silver grains over gonadotrophs from normal male rats which were fixed for electron microscopy at 0, 5, 15, 30, 60, 120 and 240 minutes after a 5-minute pulse of radioactive label was used to ascertain the location of radioactive precursor molecules.

0 minutes Tissue fixed immediately after rinsing with excess unlabeled leucine following a 5-minute pulse labeling period, and therefore in contact with the radioactive amino acid for not longer than 6 minutes in all, showed a high proportion of grains over the RER. A considerable proportion of the radioactivity observed at the earliest intervals studied was also present over the transitional

Figure 32. Dissociated adenohypophysis showing a follicular cell (FC) with microvillous processes (mvp). x16,000.

Figure 33. Dissociated adenohypophysis showing endothelial cell (EC) with microvillous processes (mvp). x11,500.


mvp
FC
32

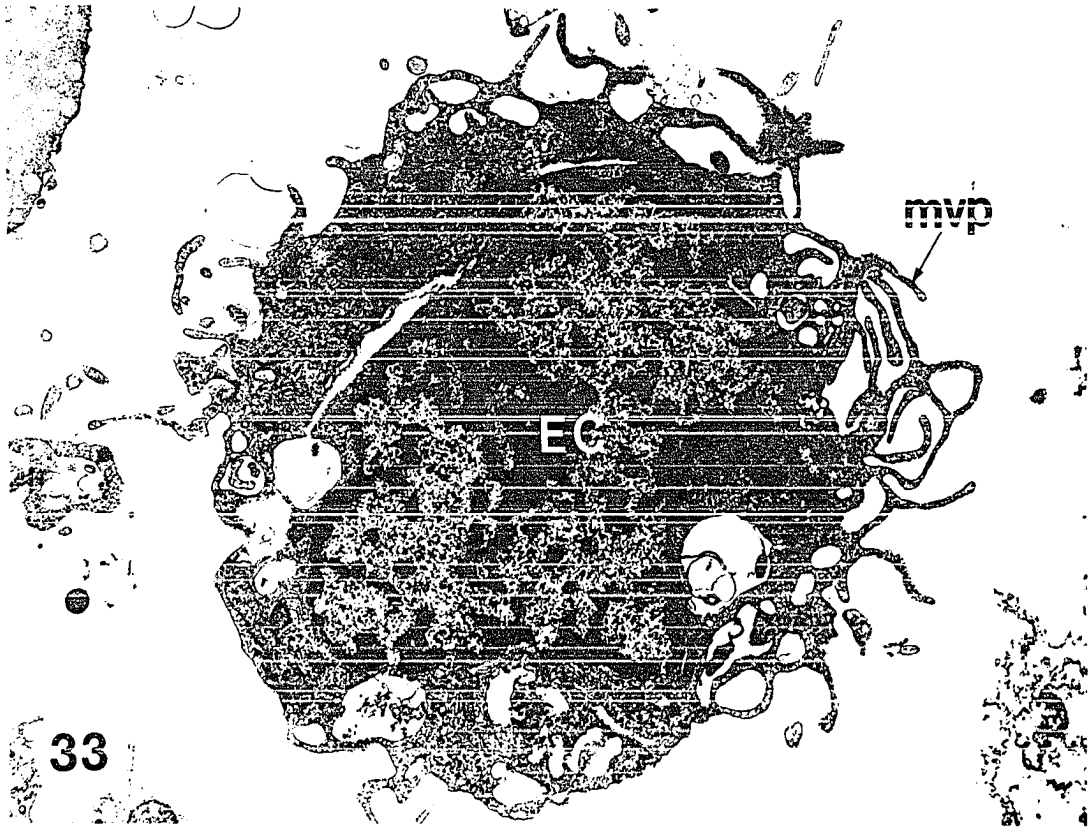
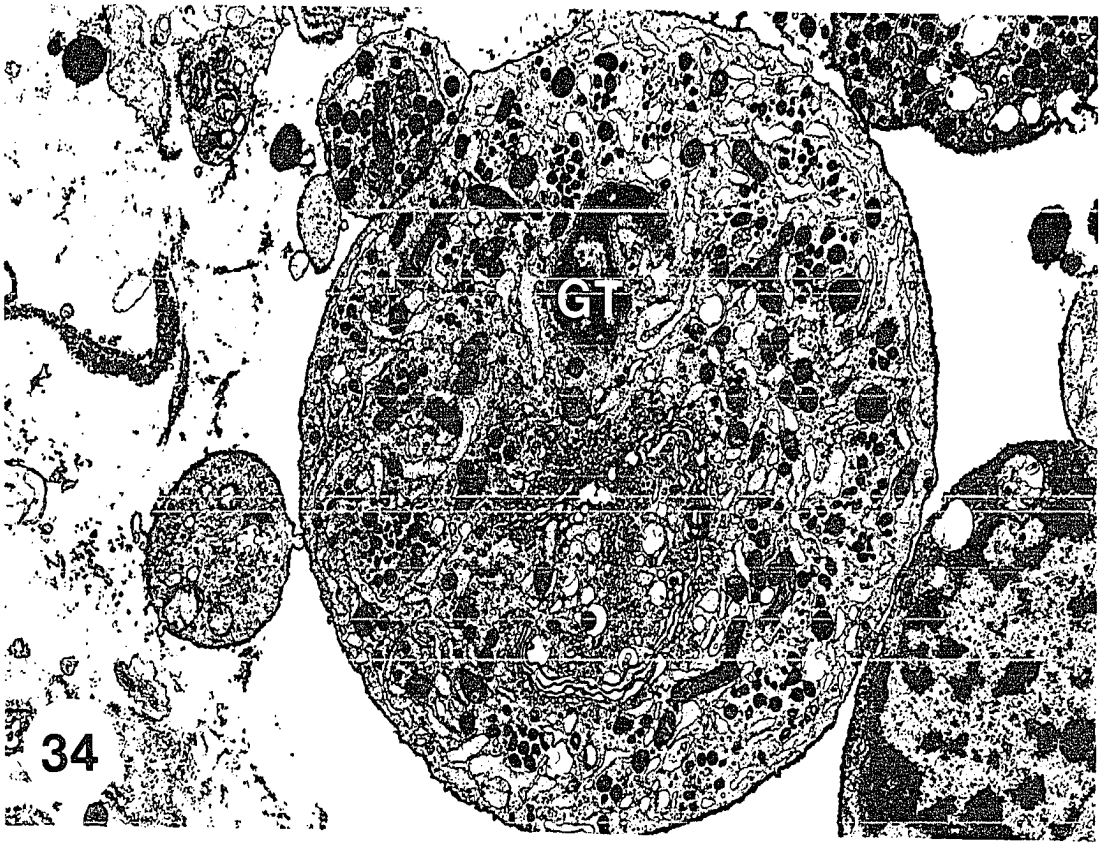

mvp
EC
33

Figure 34. Dissociated adenohypophysis 15 days after castration showing a gonadotroph (GT) with a Golgi complex (g). x9000.

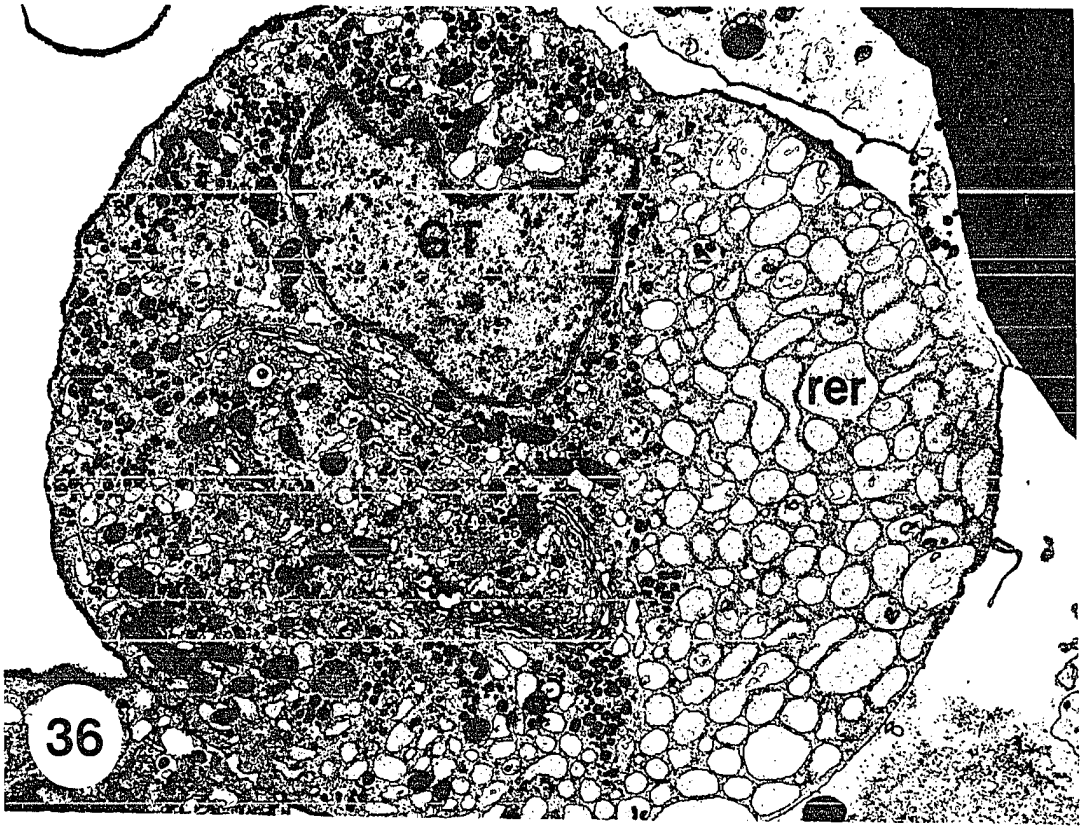
Figure 35. Cytoplasm of the gonadotroph in Figure 34 showing rough endoplasmic reticulum (rer), Golgi complex (g), immature secretory granules (isg), and vacuoles (vac). x18,000.

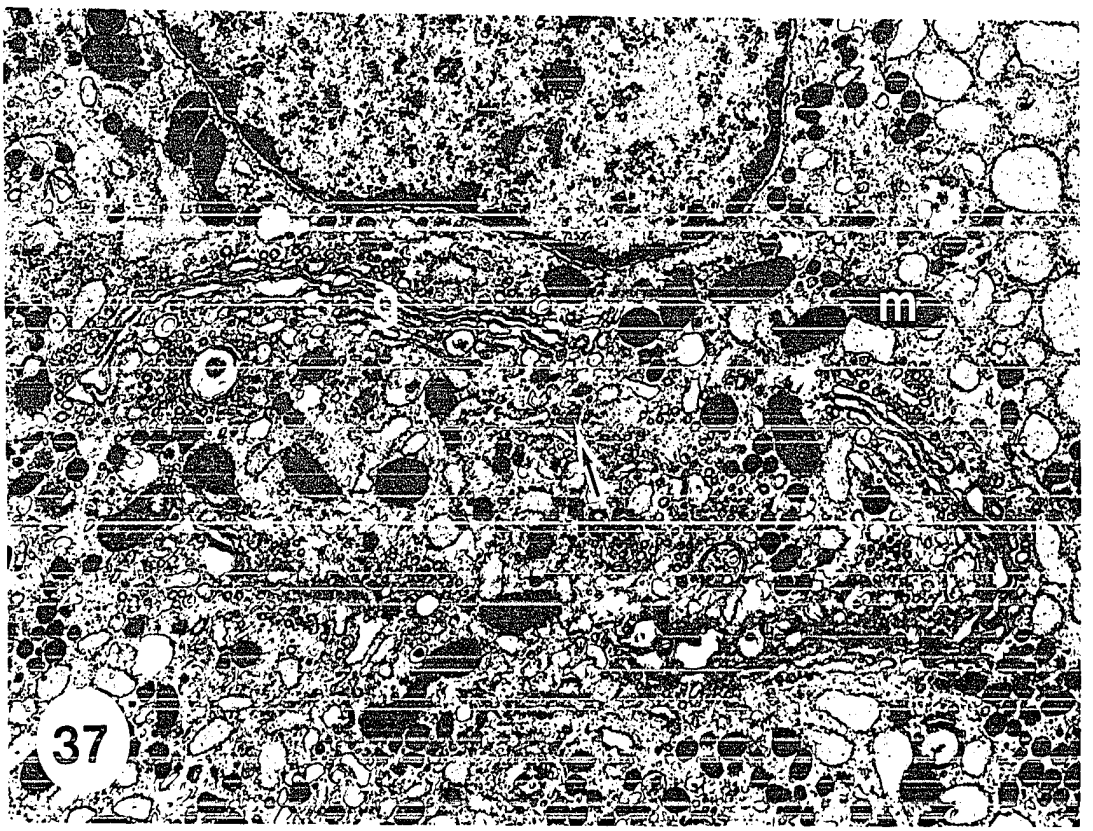

GT
34


rer
Sa
35

Figure 36. Dissociated adenohypophysis 30 days after castration showing a gonadotroph (GT), Golgi complex (g), and rough endoplasmic reticulum (rer). x9000.

Figure 37. Cytoplasm of the gonadotroph in Figure 36 showing mitochondria (m) and an immature secretory granule (isg). x18,000.


GT
rer
36


m
37

areas between the RER and the Golgi complex and in peripheral regions of the Golgi complex (Figure 38). Labeling of other organelles and, in particular, of storage granules was at a low level.

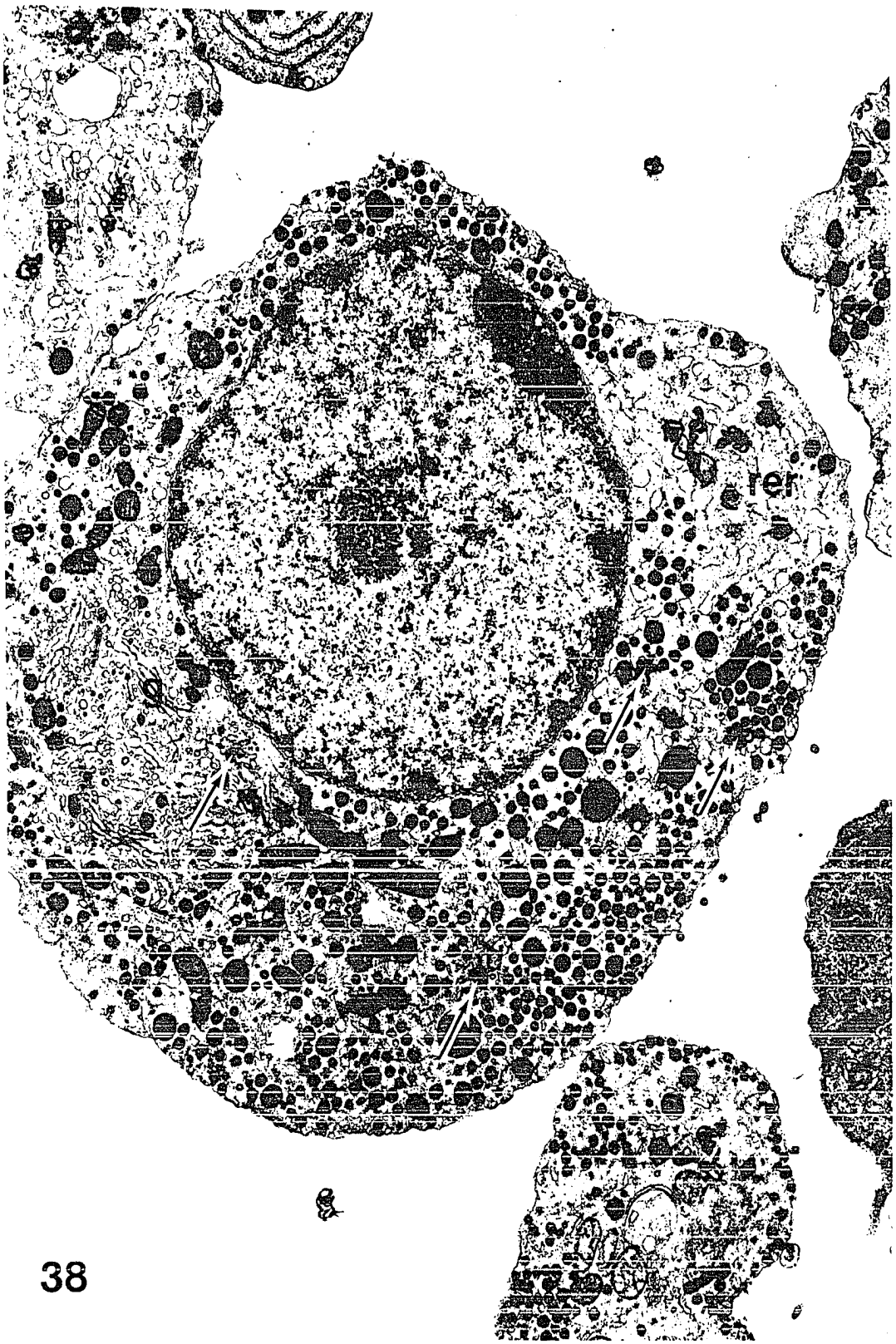
5 minutes Tissue fixed 5 minutes after the pulse and thus in contact with the label for 10 minutes showed a high concentration of silver grains over the RER and the transitional areas between the endoplasmic reticulum and the cis face of the Golgi complex (Figure 39). No significant numbers of silver grains were observed over the other organelles.

15 minutes By 15 minutes after the end of pulse labeling, a high proportion of the radioactivity was present in the transitional areas, associated with transfer vesicles on the cis side of the Golgi complex and the lamellae of the Golgi complex (Figure 40). There was a marked reduction in the profiles of silver grains over the RER (Figure 40).

30 minutes Thirty minutes after the pulse label, there was an increase in the number of silver grains over transitional membranous organelles surrounding the Golgi apparatus. Most of this activity was found on the cis face (Figure 41).

60 minutes One-fourth of all the silver grains present were over the lamellae of the Golgi complex at this time and a small increase in the proportion of labeled storage granules was evident for the first time. High levels of radioactivity remained over transitional elements of the

Figure 38. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 0 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.



38

Figure 39. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 5 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

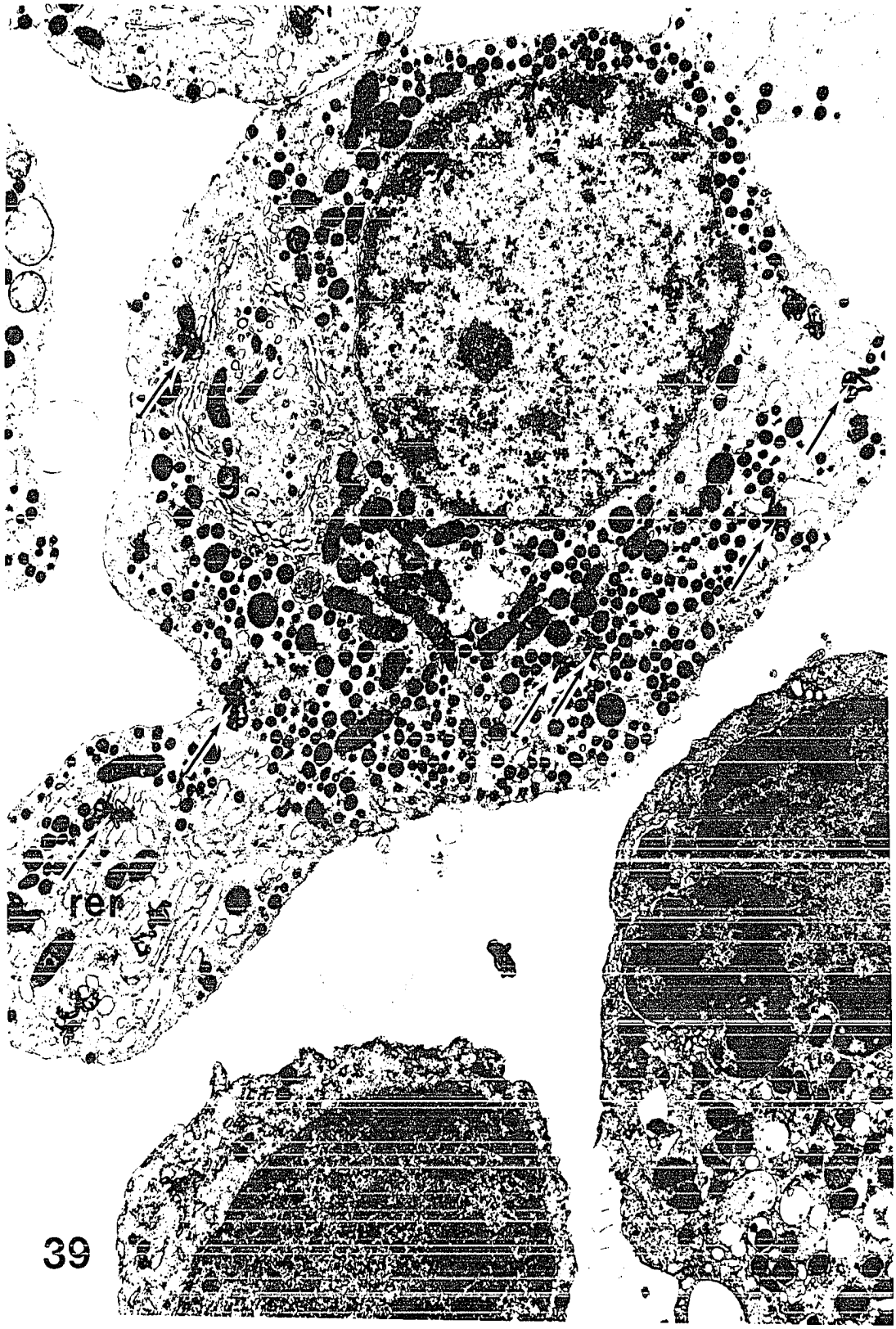

g
rer
39

Figure 40. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 15 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

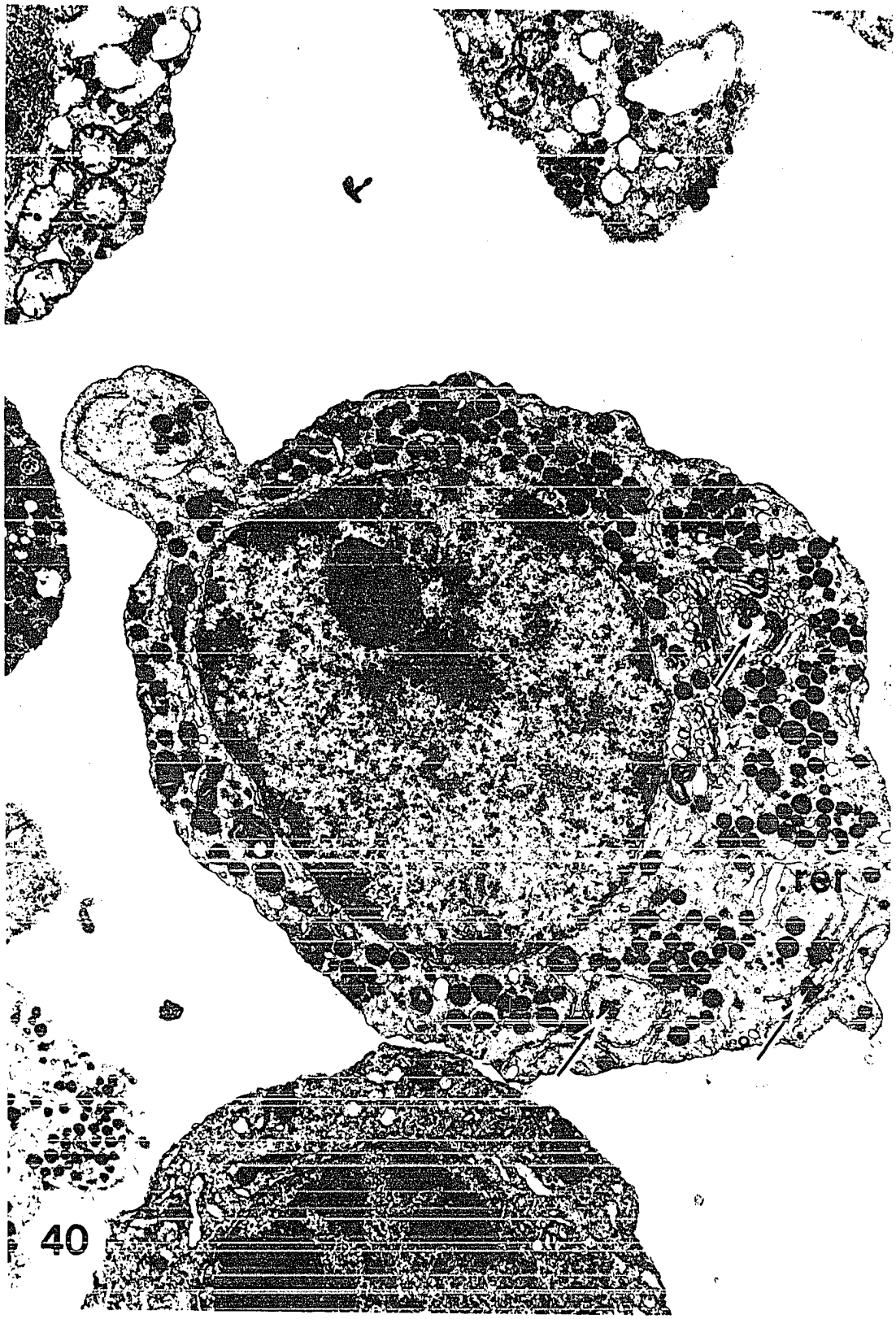

rer
40

Figure 41. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 30 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


41

cytoplasm peripheral to the Golgi complexes (Figure 42).

120 and 240 minutes The hormone storage granules were the most heavily labeled organelles at each of these times. Labeling over the Golgi complex had fallen to progressively very low levels and there appeared to be some return of radioactivity to the elements of the RER between 120 and 240 minutes (Figures 43 and 44).

Numbers of silver grains over lysosomes and mitochondria remained at consistently low levels at each of the time periods studied. A fairly constant degree of labeling within the nucleus at every time interval studied was noted.

Pulse labeling with leucine-^{3}H (15 days after castration)

0 minutes Immediately after the pulse, the silver grains were found over the cisternae of the RER (Figure 45). Relatively few silver grains were found over transitional areas of the Golgi complex (Figure 45).

5 minutes A greater number of silver grains were observed over the transitional elements of the Golgi complex as well as the cisternae of the RER (Figure 46). Labeling of other organelles was at a very low level.

15 minutes More activity was noted over the cis and trans elements of the Golgi complex with some grains over peripheral regions of the Golgi complex (Figure 47).

30 minutes Thirty minutes after the pulse, the small transitional vesicles of the SER and the fenestrated cis and

Figure 42. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 60 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

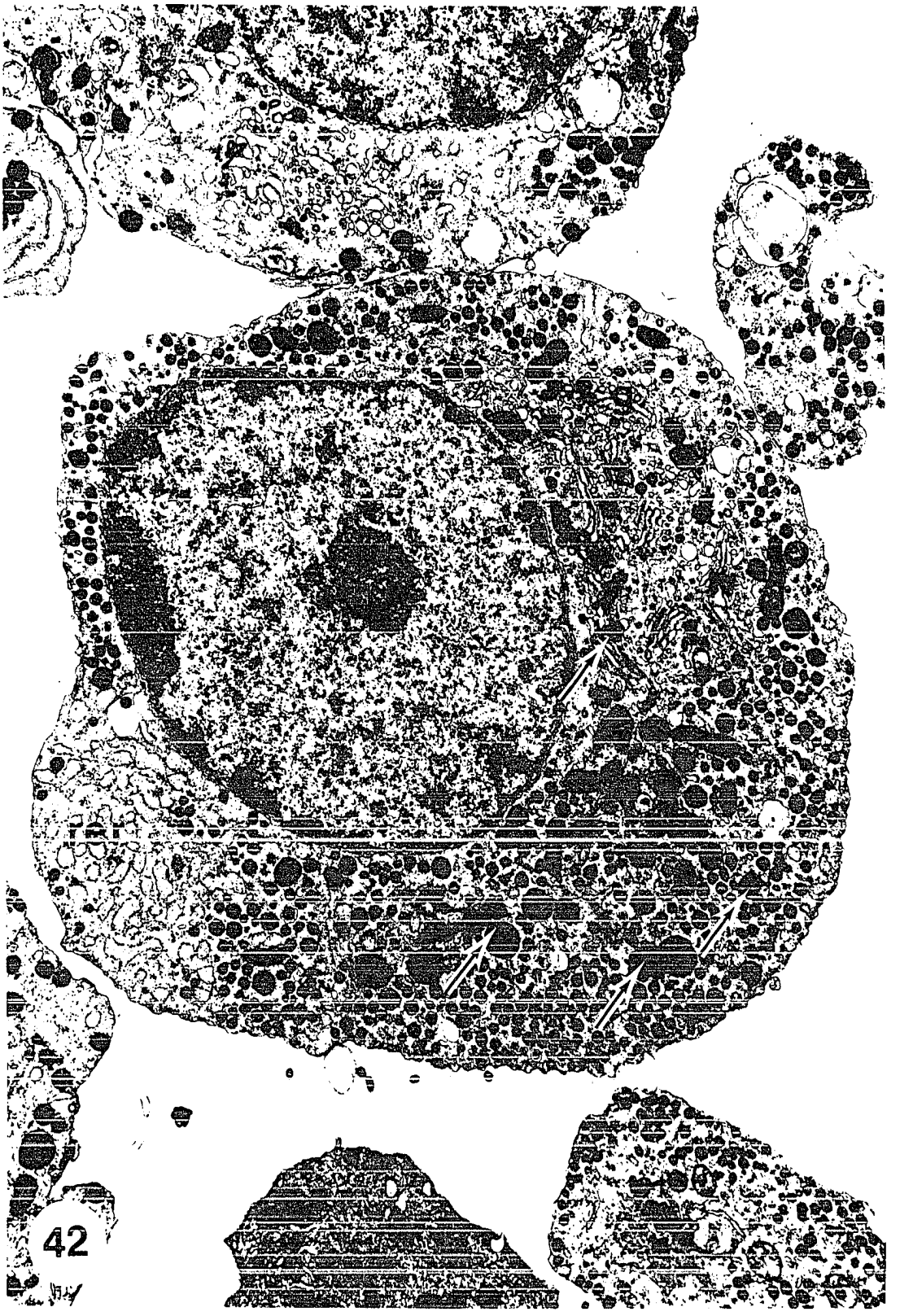

42

Figure 43. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 120 minutes. Arrows indicate silver grains. x11,500.

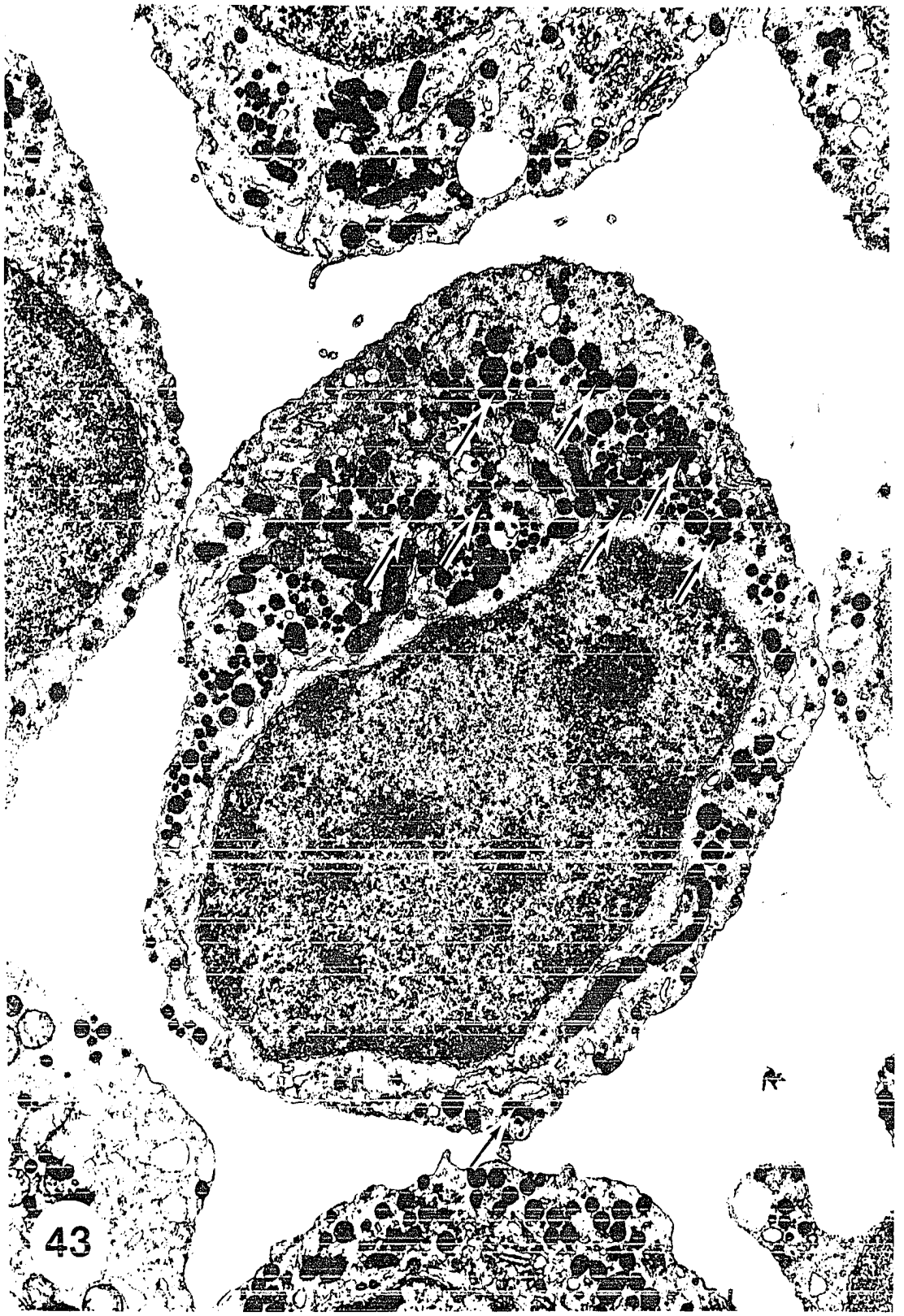

43

Figure 44. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with leucine-^{3}H and incubated for 240 minutes, showing rough endoplasmic reticulum (rer). Arrows indicate silver grains. x11,500.

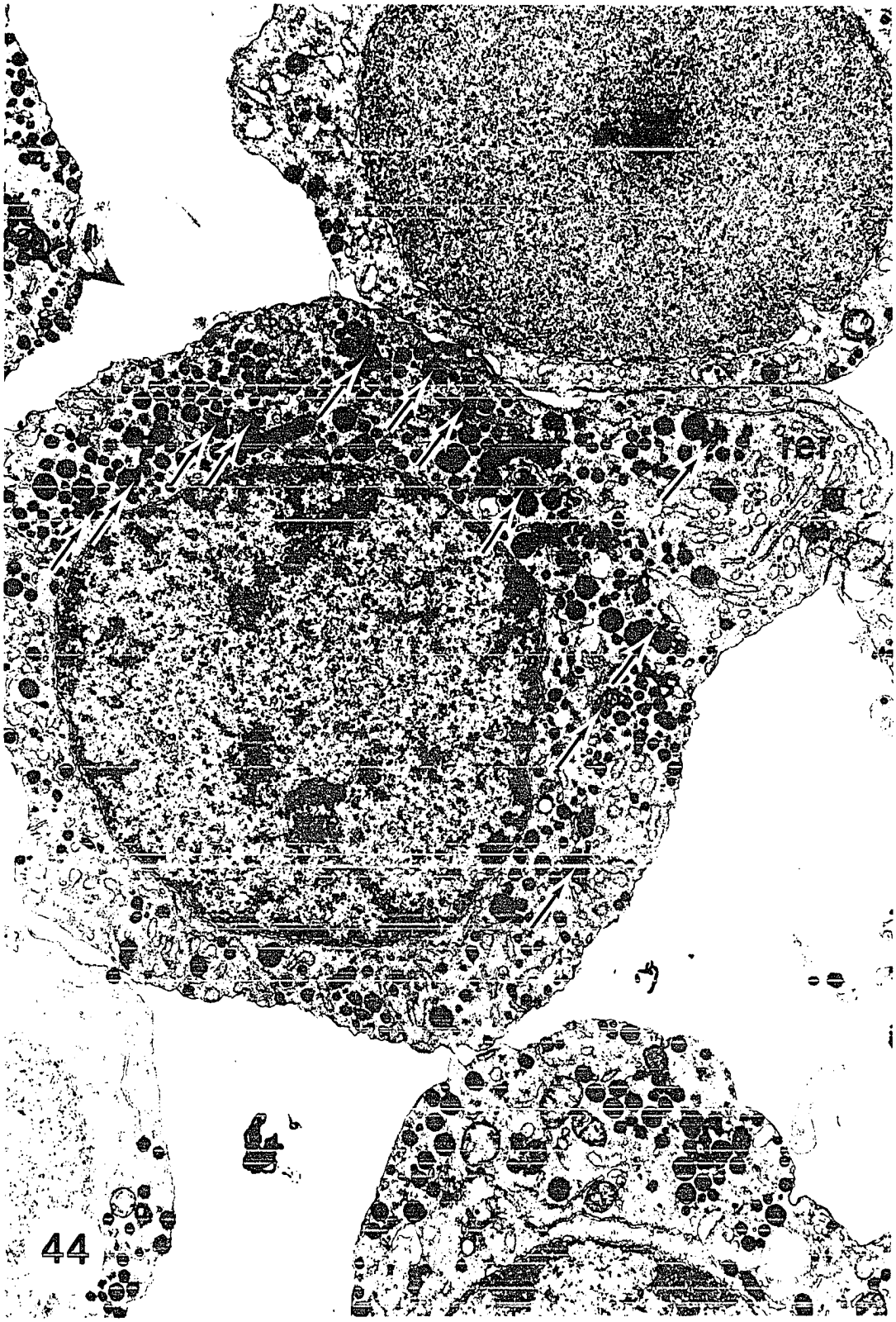

ter
44

Figure 45. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 0 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


rer
45

Figure 46. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 5 minutes showing rough endoplasmic reticulum (rer). Arrow indicates silver grain. x11,500.

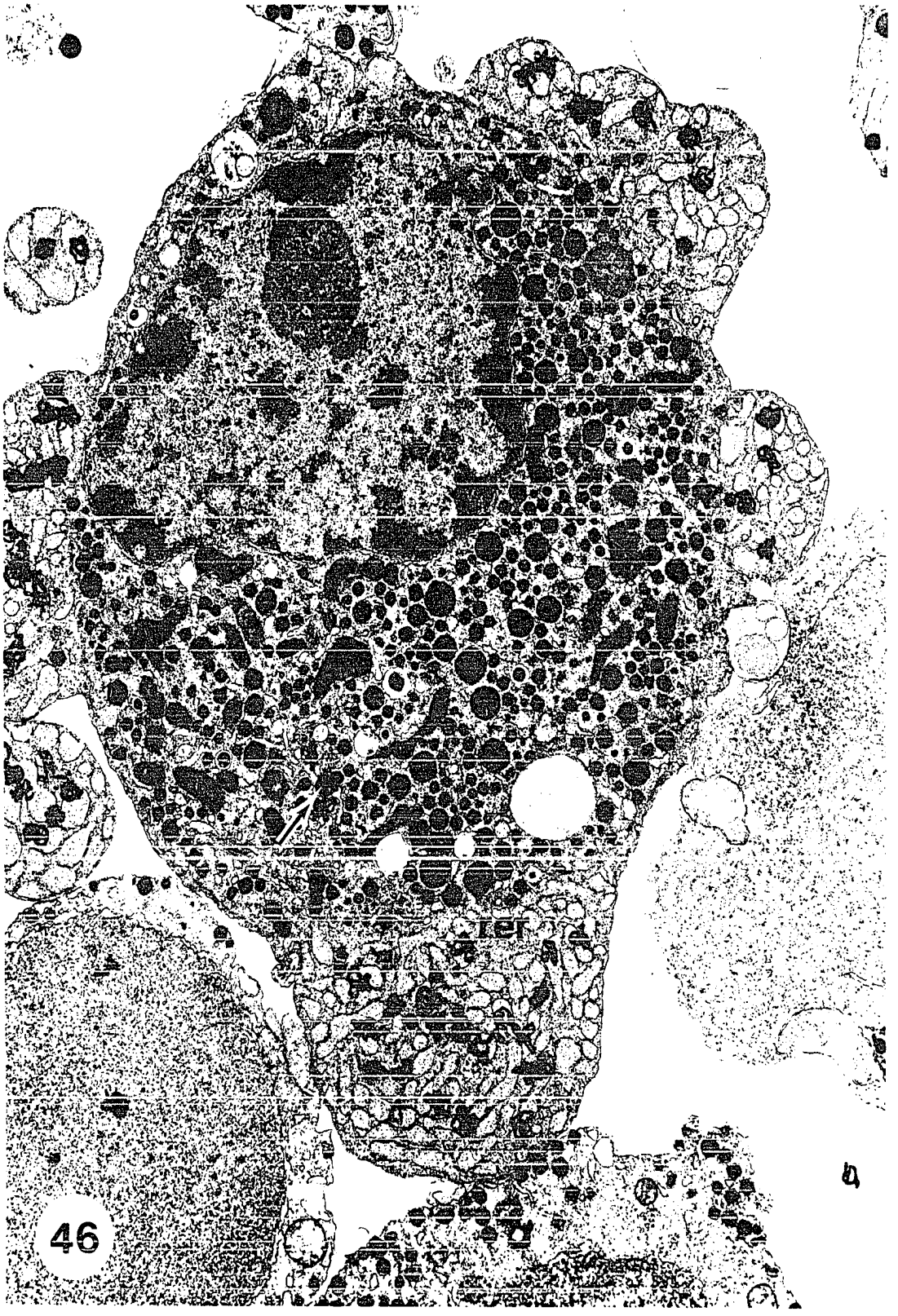
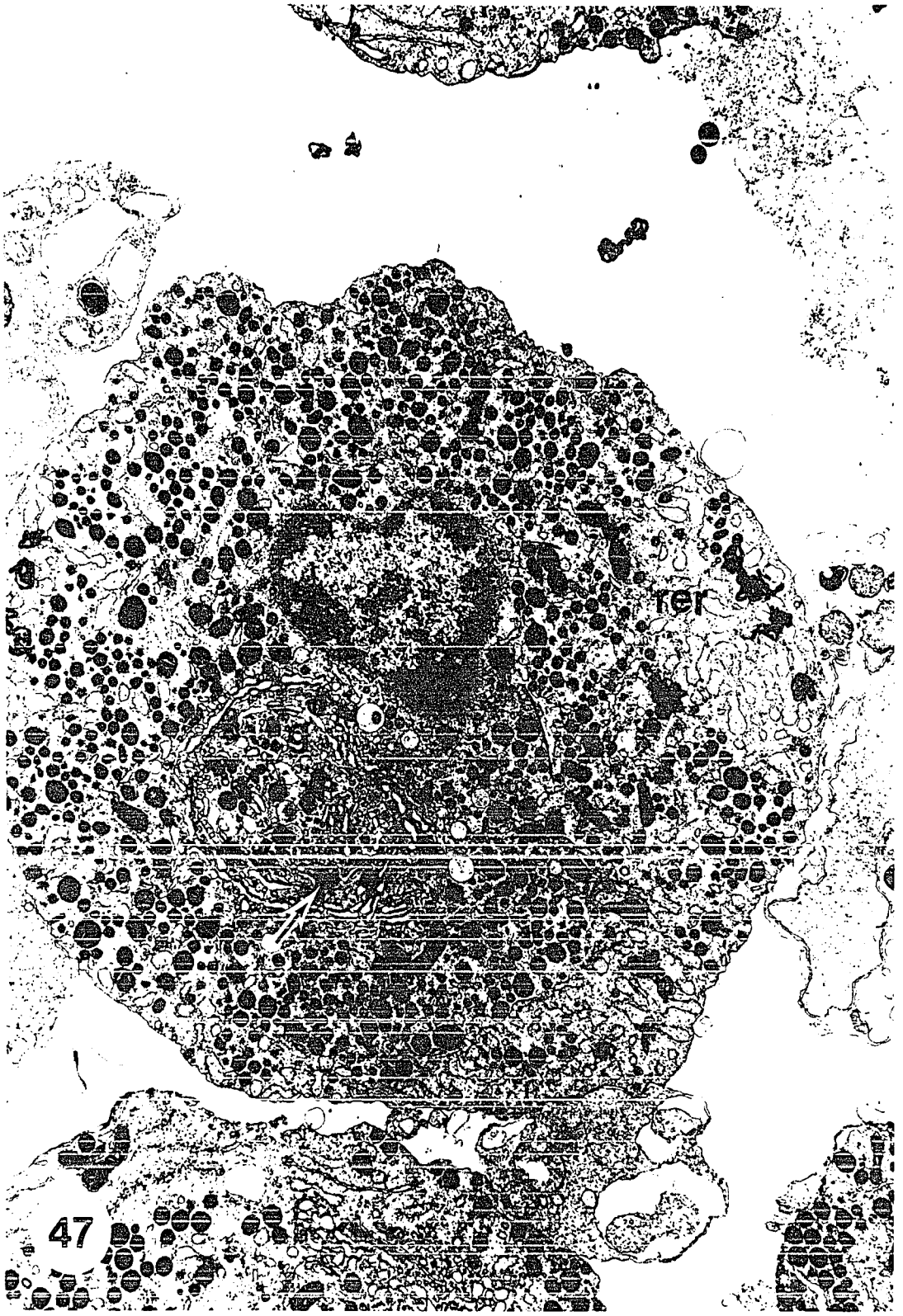

46

Figure 47. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 15 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrow indicates silver grain. x11,500.


rer
47

the trans lamellae had silver grains over them (Figure 48). There were larger numbers of silver grains over the peripheral tubular and vesicular elements of the Golgi complex (Figure 48).

60 minutes One hour after the pulse, the silver grains were concentrated over the Golgi complex (Figure 49). There were silver grains over the fenestrated middle elements of the Golgi complex and the tubular and vesicular elements of the SER near the trans face of the Golgi complex (Figure 49). Some labeling was observed over immature secretory granules in the inner cisternal elements of the trans face of the Golgi complex (Figure 49).

120 and 240 minutes The label was now sequestered in the interstitial cell stimulating hormone containing secretory granules (Figures 50 and 51). However some silver grains remained over elements of the RER and the Golgi complex (Figures 50 and 51). Again the nuclear material had a significant number of silver grains over it while the mitochondria and lysosomes had very low levels of activity (Figures 50 and 51).

Pulse labeling with leucine-^{3}H (30 days after castration)

0 minutes The silver grains were found over the large cisternae of the RER immediately following the pulse (Figure 52). There was also some labeling over the cis cisternae of the Golgi complex and the vesicular elements of

Figure 48. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 30 minutes, showing rough endoplasmic reticulum (rer). Arrows indicate silver grains. x11,500.


rer
48

Figure 49. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 60 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

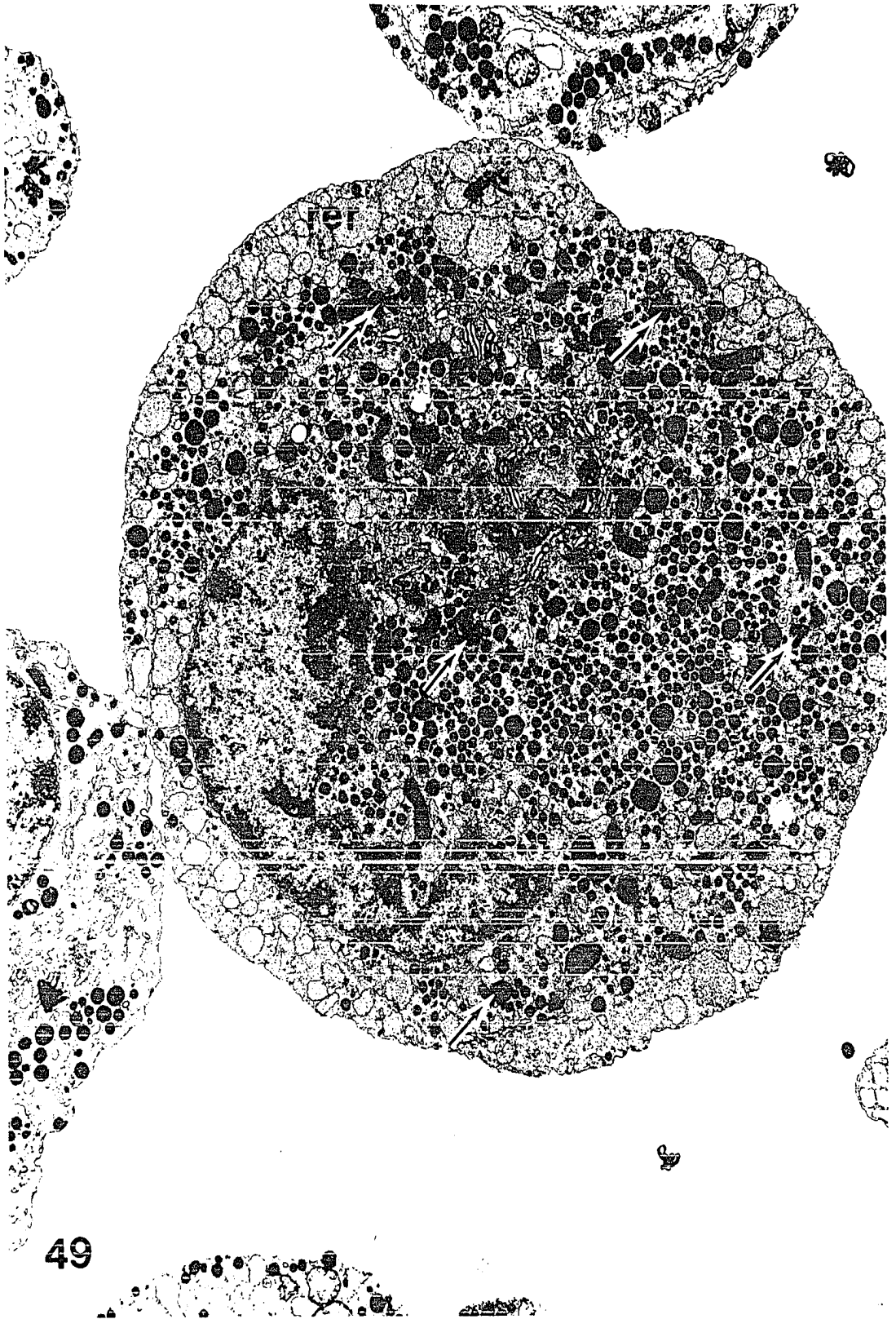

rer
49

Figure 50. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 120 minutes, showing Golgi complex (g). Arrows indicate silver grains. x11,500.

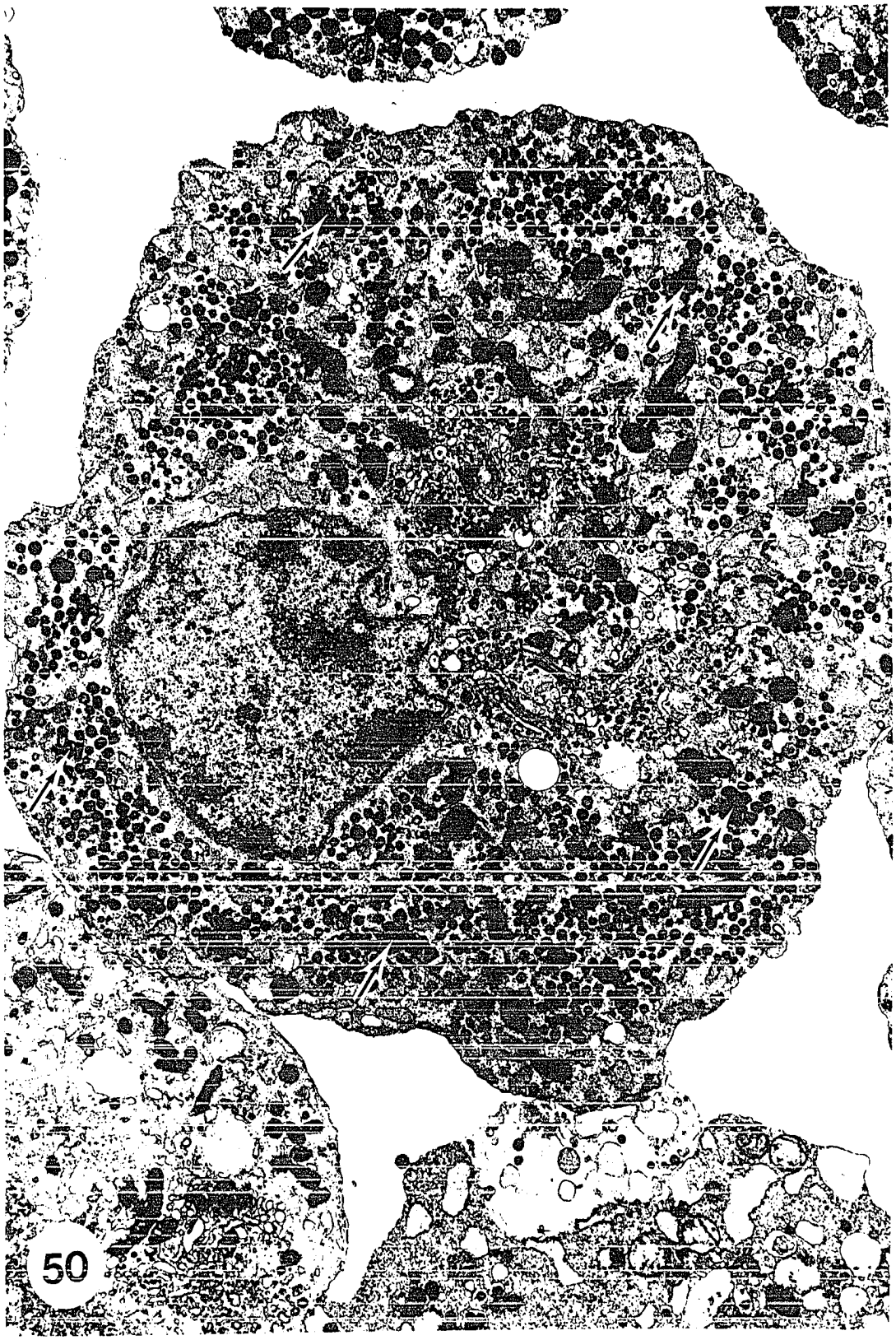
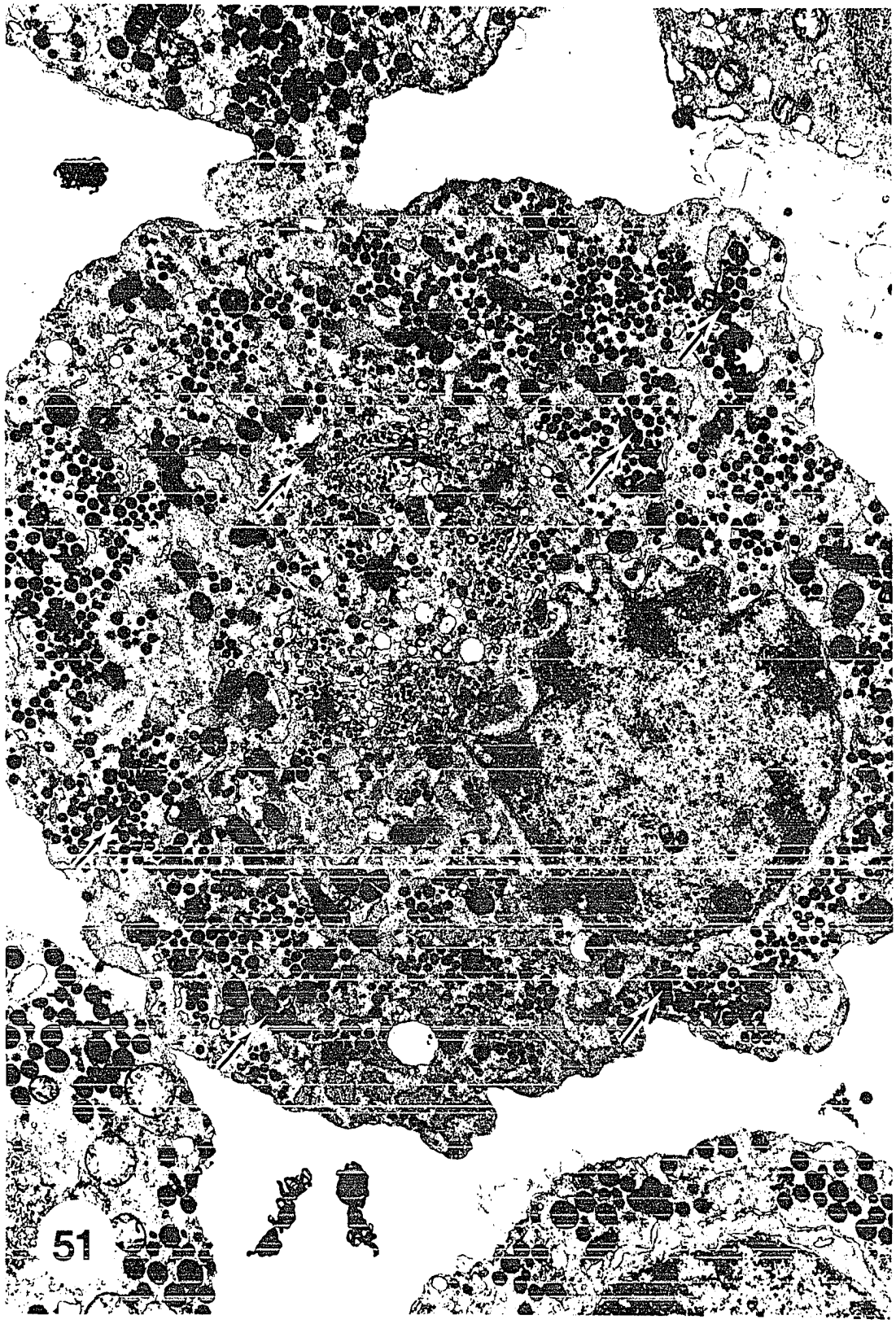

50

Figure 51. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with leucine-^{3}H and incubated for 240 minutes, showing Golgi complex (g). Arrows indicate silver grains. x11,500.


51

the transitional endoplasmic reticulum (Figure 52).

5 minutes Tissue fixed five minutes after the pulse and therefore in contact with the radioactive label no longer than ten minutes showed an accumulation of silver grains over the dilated cisternae and membranes of the RER (Figure 53). There were also a few silver grains over the transitional elements of the endoplasmic reticulum near the cis elements of the Golgi complex (Figure 53).

15 minutes More activity was observed over the transitional elements of the endoplasmic reticulum and the cis components of the Golgi complex (Figure 54). There were some silver grains over peripheral Golgi elements and some middle lamellae of the Golgi body (Figure 54). However some grains were observed over vesicles in the periphery and trans areas of the Golgi complex (Figure 54).

30 minutes There is an increased number of silver grains over the membrous elements of the cis region of the Golgi complex (Figure 55). However some grains are observed over vesicles in the periphery and trans areas of the Golgi complex (Figure 55).

60 minutes At this time the silver grains were concentrated over the lamellae of the Golgi complex with many grains over the distended and dilated outer Golgi elements (Figure 56). There were some silver grains over the cisternal and tubular elements of the smooth endoplasmic reticulum adjacent to the trans face of the Golgi body (Figure 56).

Figure 52. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 0 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrow indicates silver grain. x11,500.


52

Figure 53. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 5 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

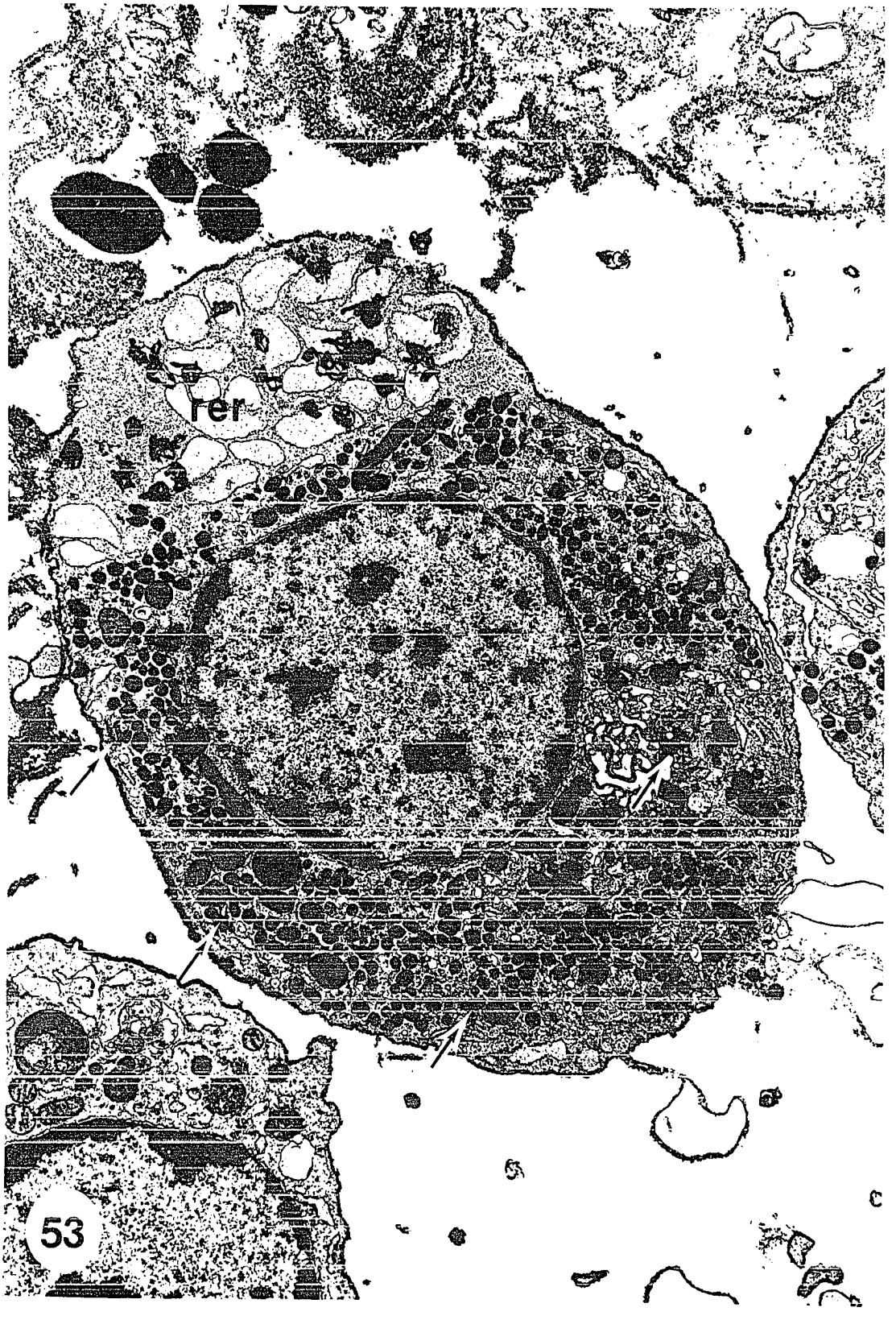

rer
53

Figure 54. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 15 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

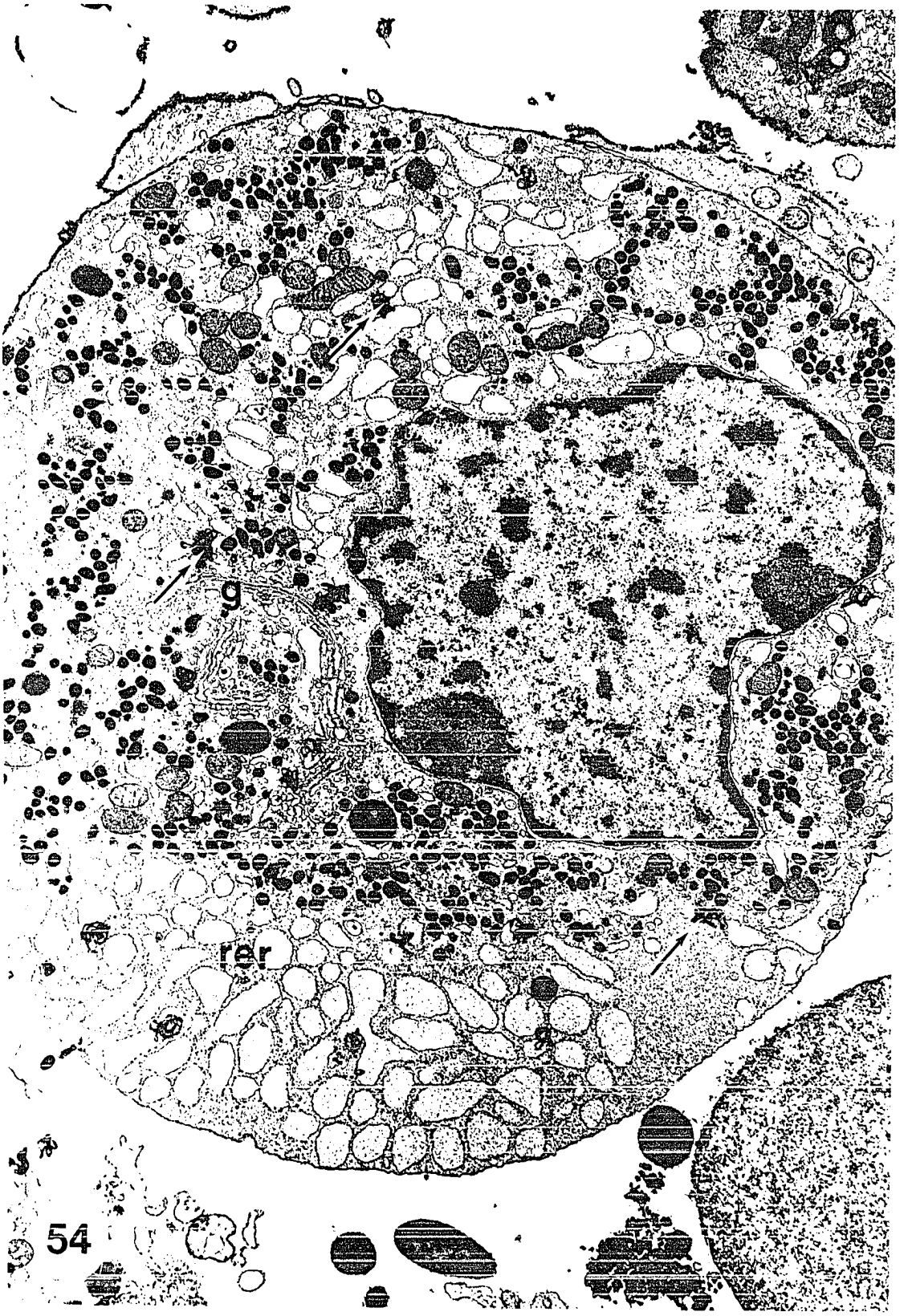

g
rer
54

Figure 55. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 30 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

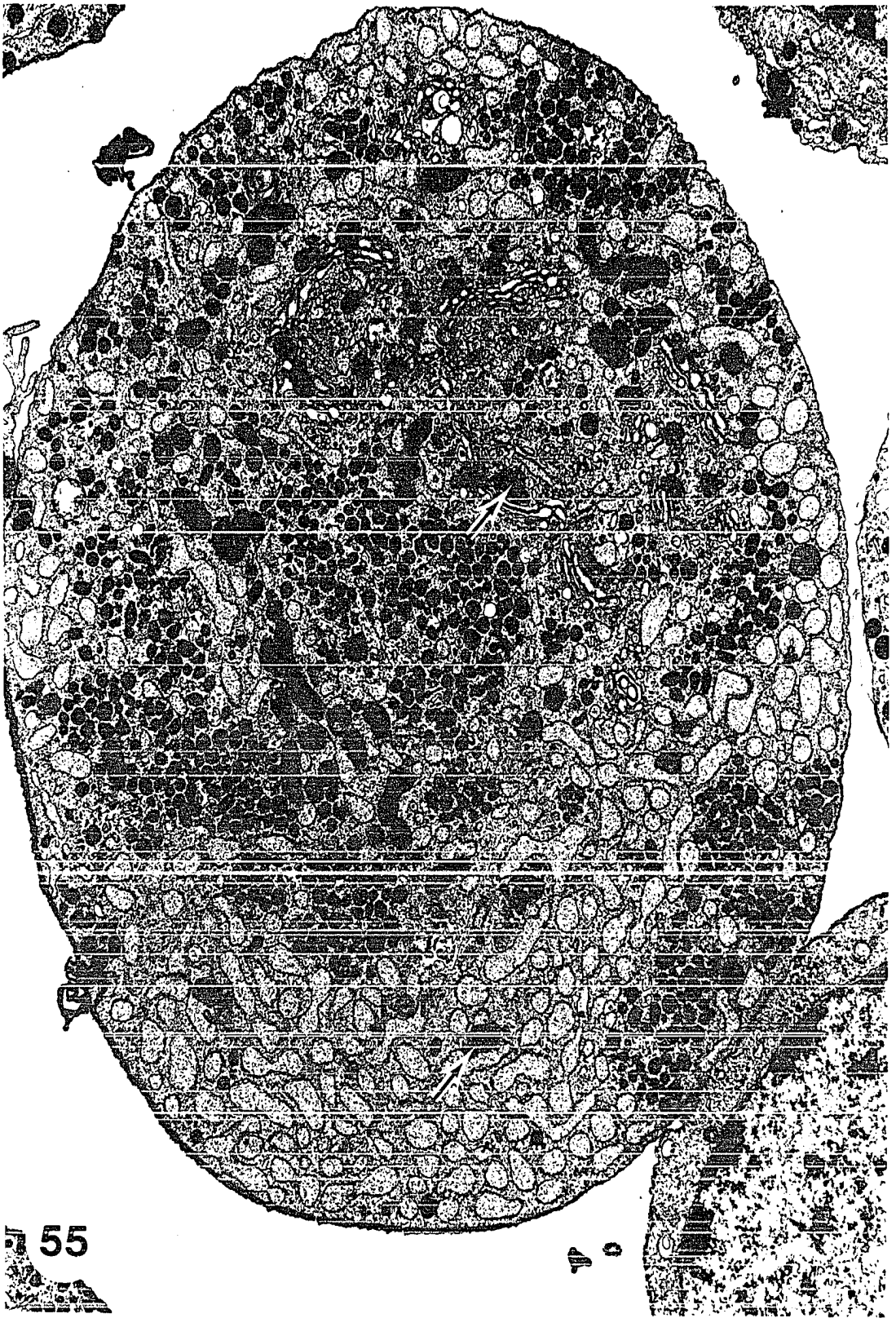

55

Figure 56. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 60 minutes showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


56

120 and 240 minutes The hormone containing granules now show nearly all of the activity with some grains over the tubular and cisternal elements of the Golgi complex (Figures 57 and 58). Some activity remained within the intervening portions of the synthetic elements of the cell (Figures 57 and 58).

Pulse labeling with mannose-^{3}H (normal male rats)

0 minutes Tissue fixed immediately after the 5-minute pulse exposure to mannose-^{3}H showed most of the silver grains over the RER (Figure 59). It was difficult to determine whether these grains were located over membranes or the content of the cisternae.

5 minutes A larger number of silver grains were found over the RER and very little activity was observed over the Golgi complex (Figure 60).

15 minutes More silver grains were over the rough and transitional endoplasmic reticulum (Figure 61). There was little activity in the cisternal elements of the Golgi complex.

30 minutes By thirty minutes, the activity had shifted to the transitional elements of the endoplasmic reticulum near the cis face of the Golgi complex and the Golgi complex (Figure 62). Some silver grains were over the cisternal elements of the Golgi complex (Figure 62). There was very little activity over the mature secretory granules.

Figure 57. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 120 minutes, showing rough endoplasmic reticulum (rer). Arrows indicate silver grains. x11,500.

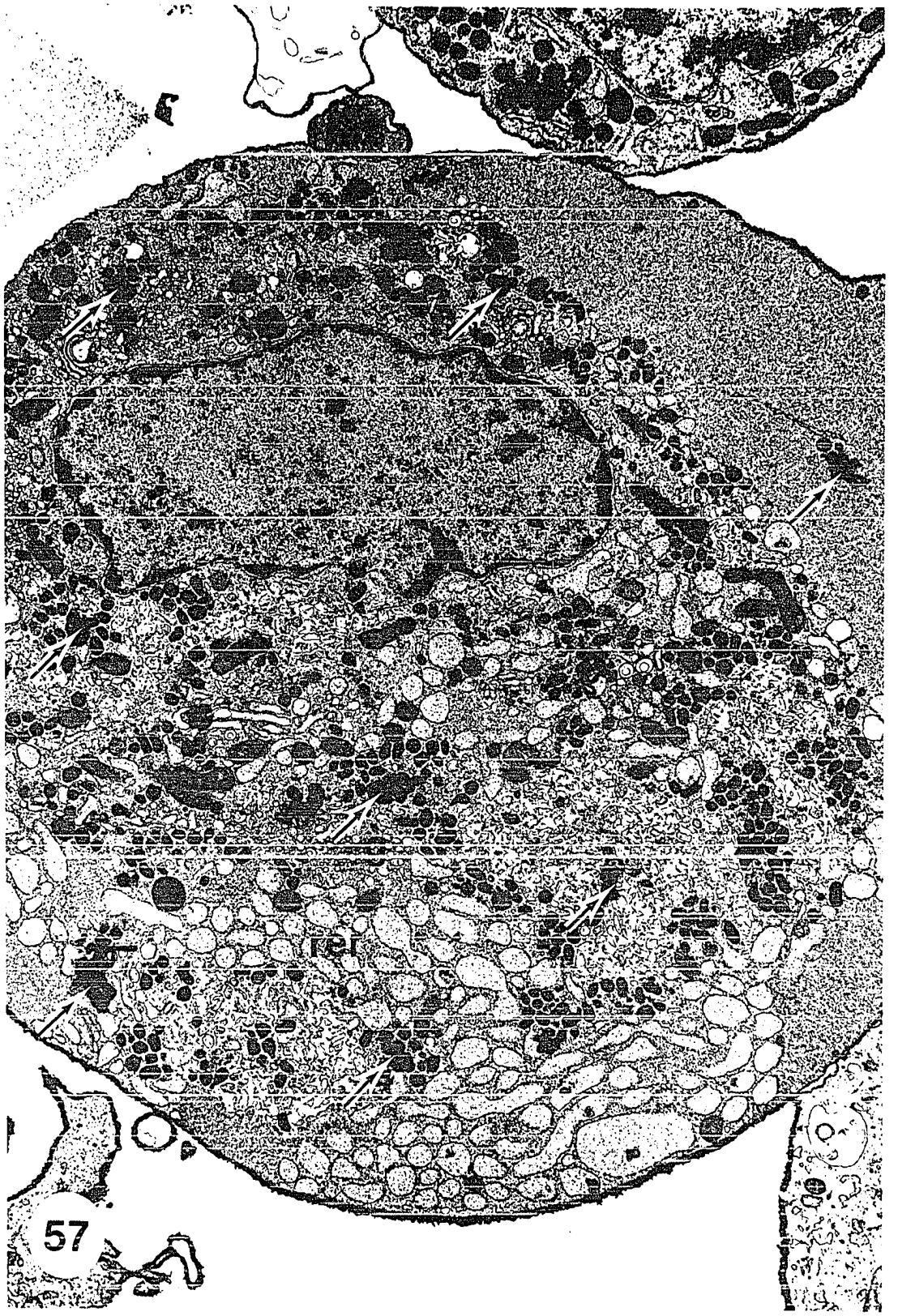

rer
57

Figure 58. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with leucine-^{3}H and incubated for 240 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


58

Figure 59. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 0 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

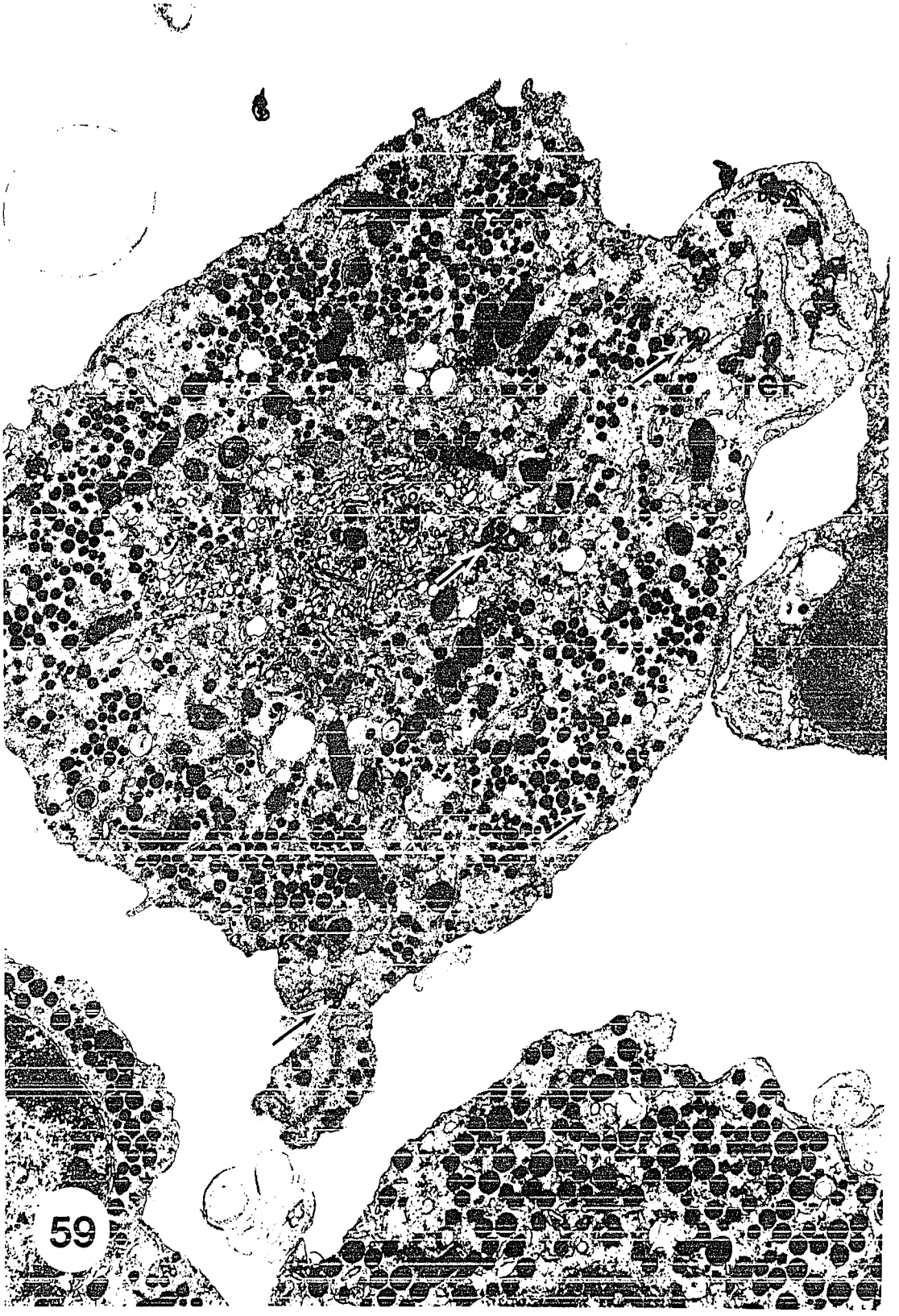

TCT
59

Figure 60. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 5 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

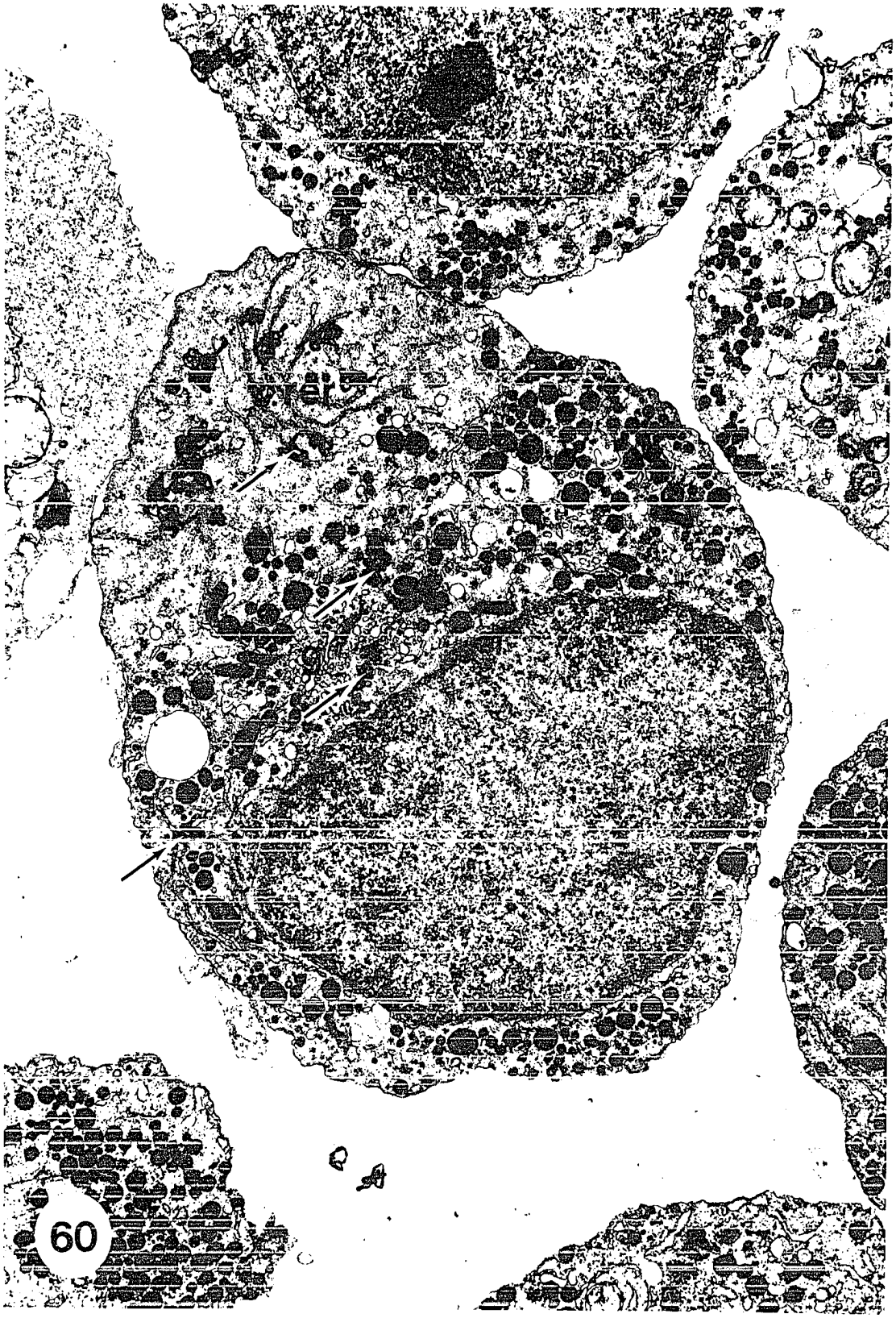

60

Figure 61. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 15 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

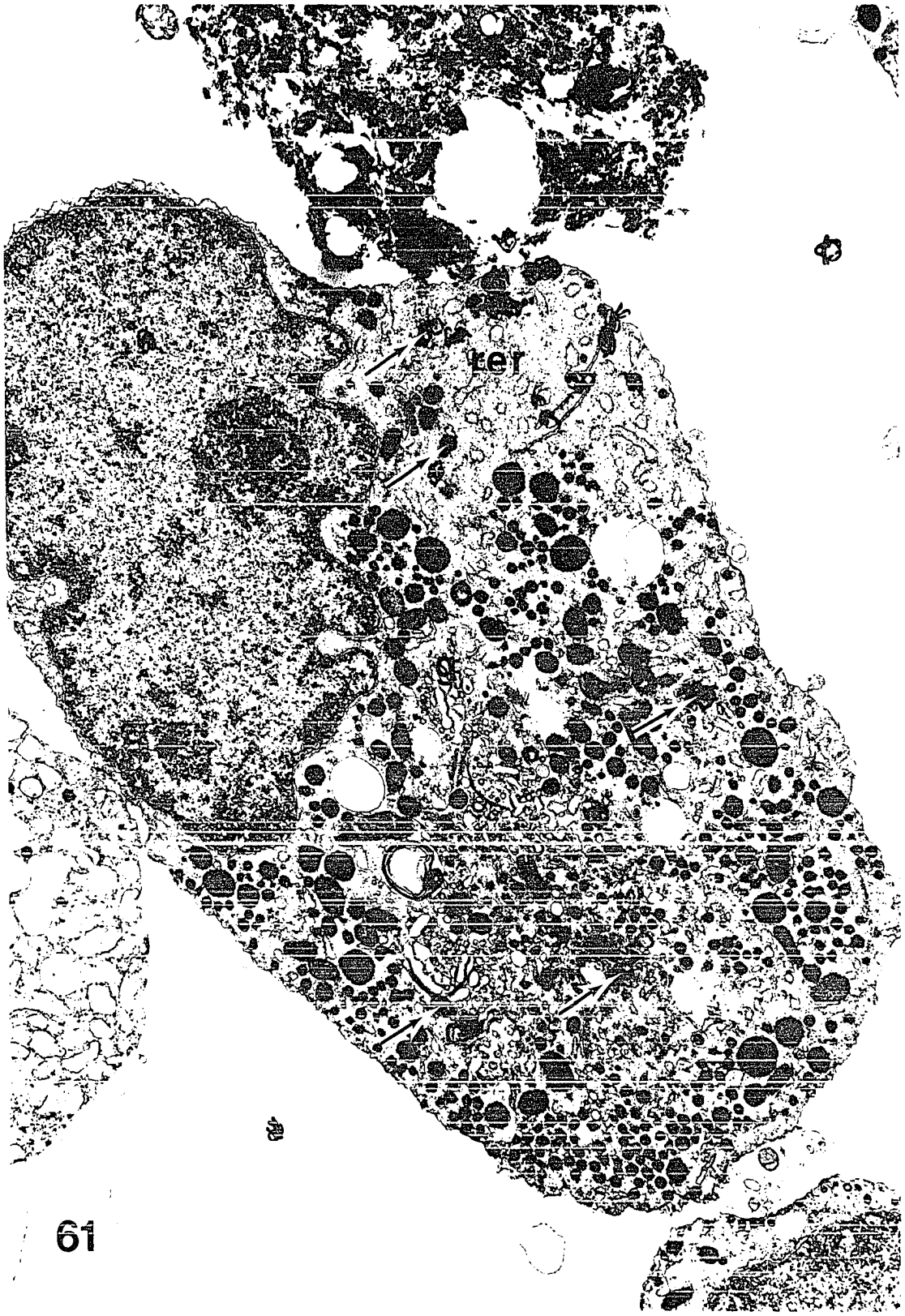
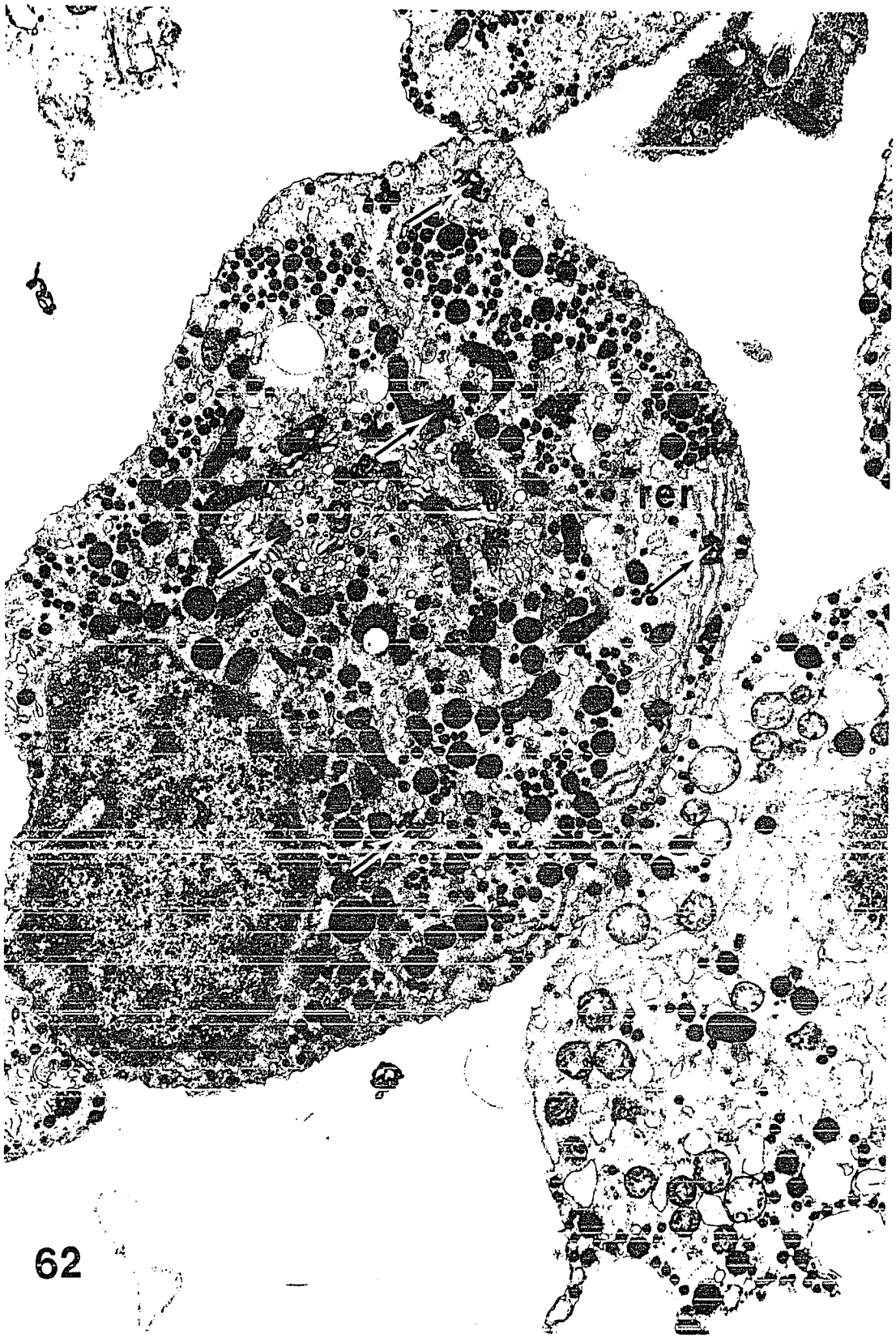

rer
61

Figure 62. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 30 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


rer
62

60 minutes After one hour the activity in the Golgi complex and its associated organelles had begun to peak (Figure 63). There were silver grains over outer middle and inner cisternal elements (Figure 63). Silver grains were also present over peripheral tubular and fenestrated cisternae (Figure 63). Activity had diminished in the RER.

120 and 240 minutes By 2 or 4 hours the activity had peaked in the Golgi complex and mature secretory granules had silver grains over them (Figures 64 and 65). Some silver grains were observed over trans elements of the Golgi complex and forming or immature secretory granules (Figures 64 and 65).

Pulse labeling with mannose-^{3}H (15 days after castration)

0 minutes Immediately following the initial pulse, the dilated cisternae of RER showed activity (Figure 66). The remainder of the cell had very little activity.

5 minutes By this time the number of silver grains over the RER had increased (Figure 67).

15 minutes By this time the silver grains were found on the cis face of the Golgi complex (Figure 68). Some activity remained in the RER and transitional elements of the ER (Figure 68).

30 minutes Thirty minutes following the pulse, the number of silver grains over the rough and smooth endoplasmic reticulum had peaked and some grains were observed over

Figure 63. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 60 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

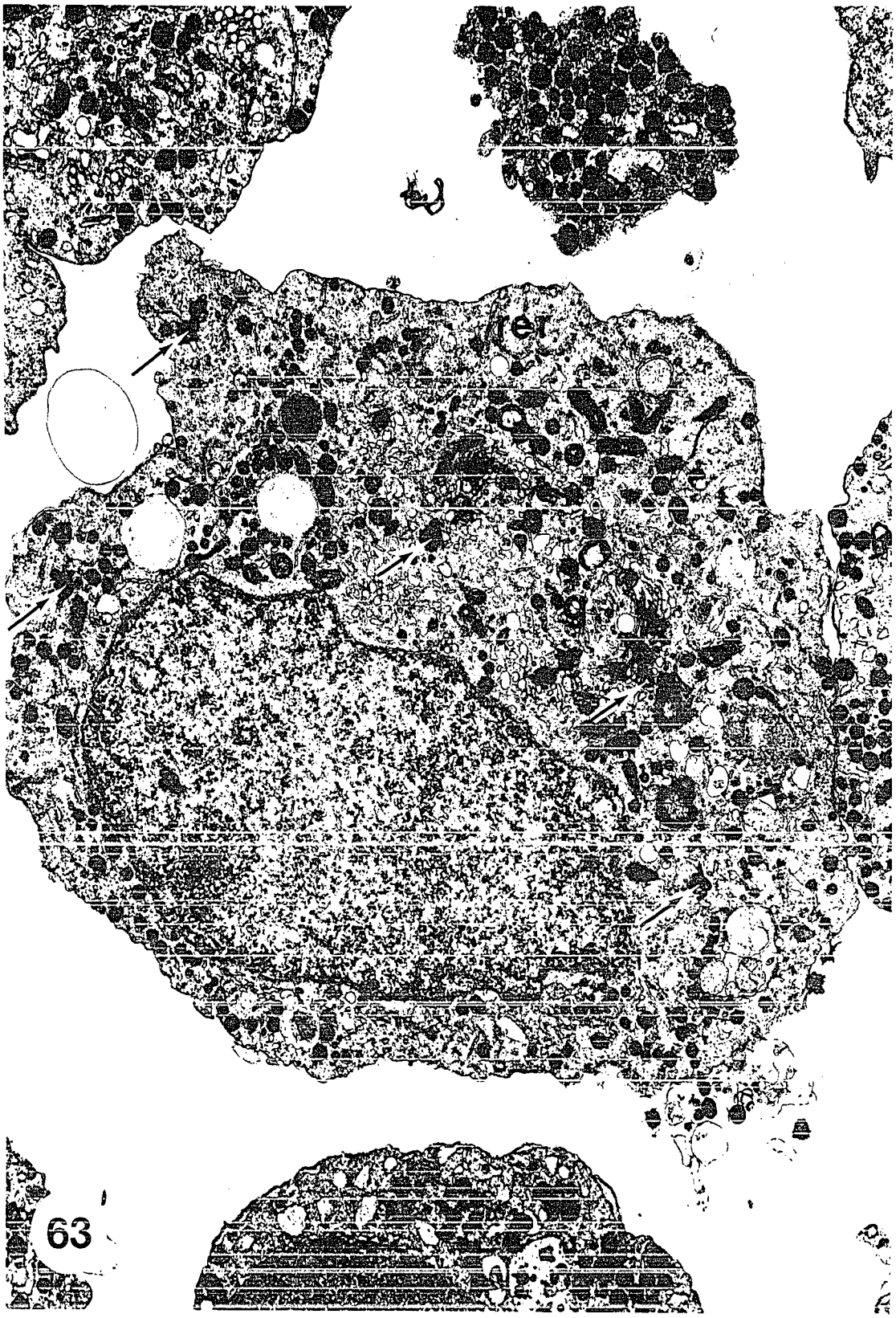
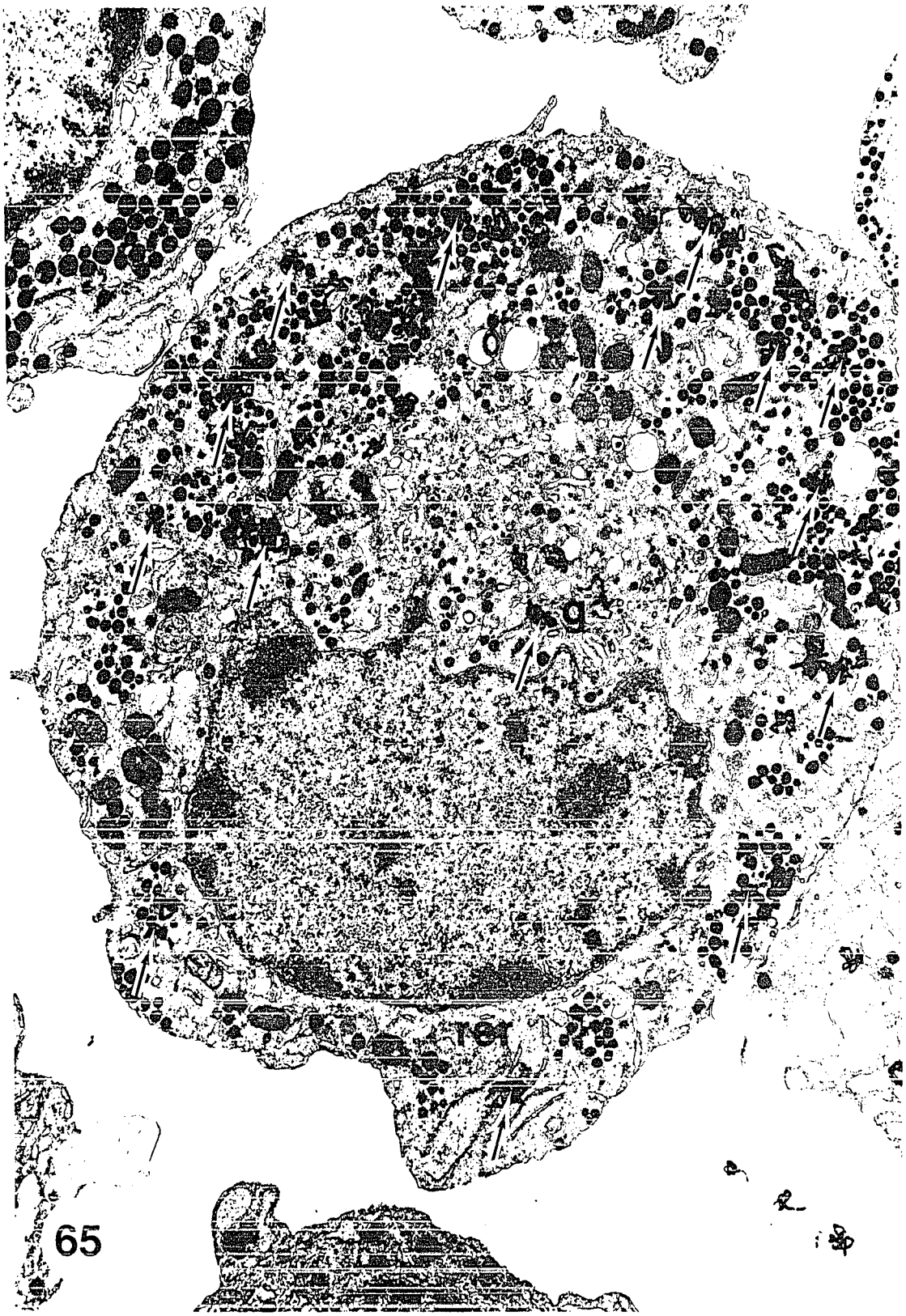

rer
63

Figure 64. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 120 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


g
rer
64

Figure 65. Autoradiograph of a gonadotroph from dissociated adenohypophysis pulse labeled with mannose-^{3}H and incubated for 240 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.



65

Figure 66. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 0 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

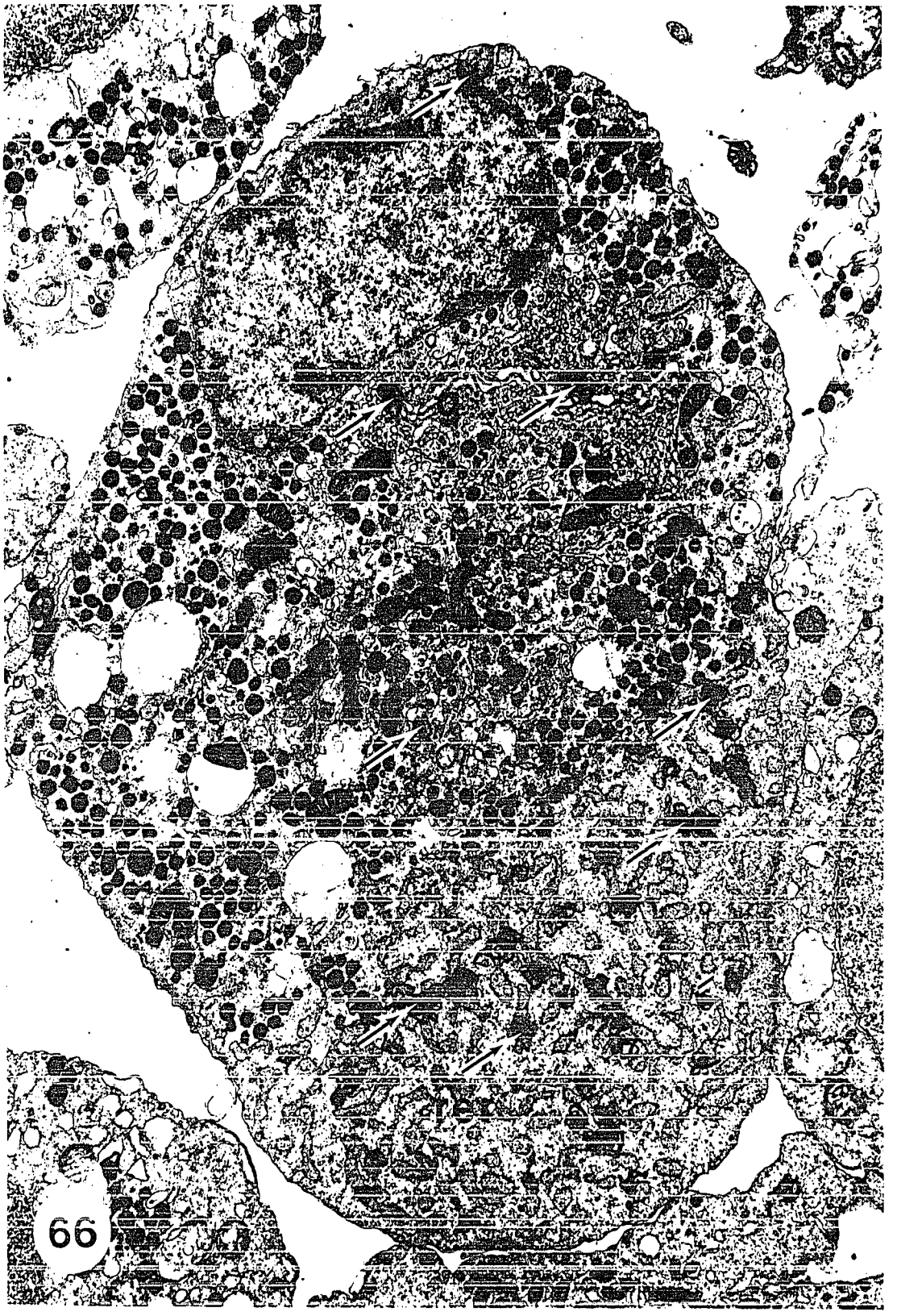

66

Figure 67. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 5 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

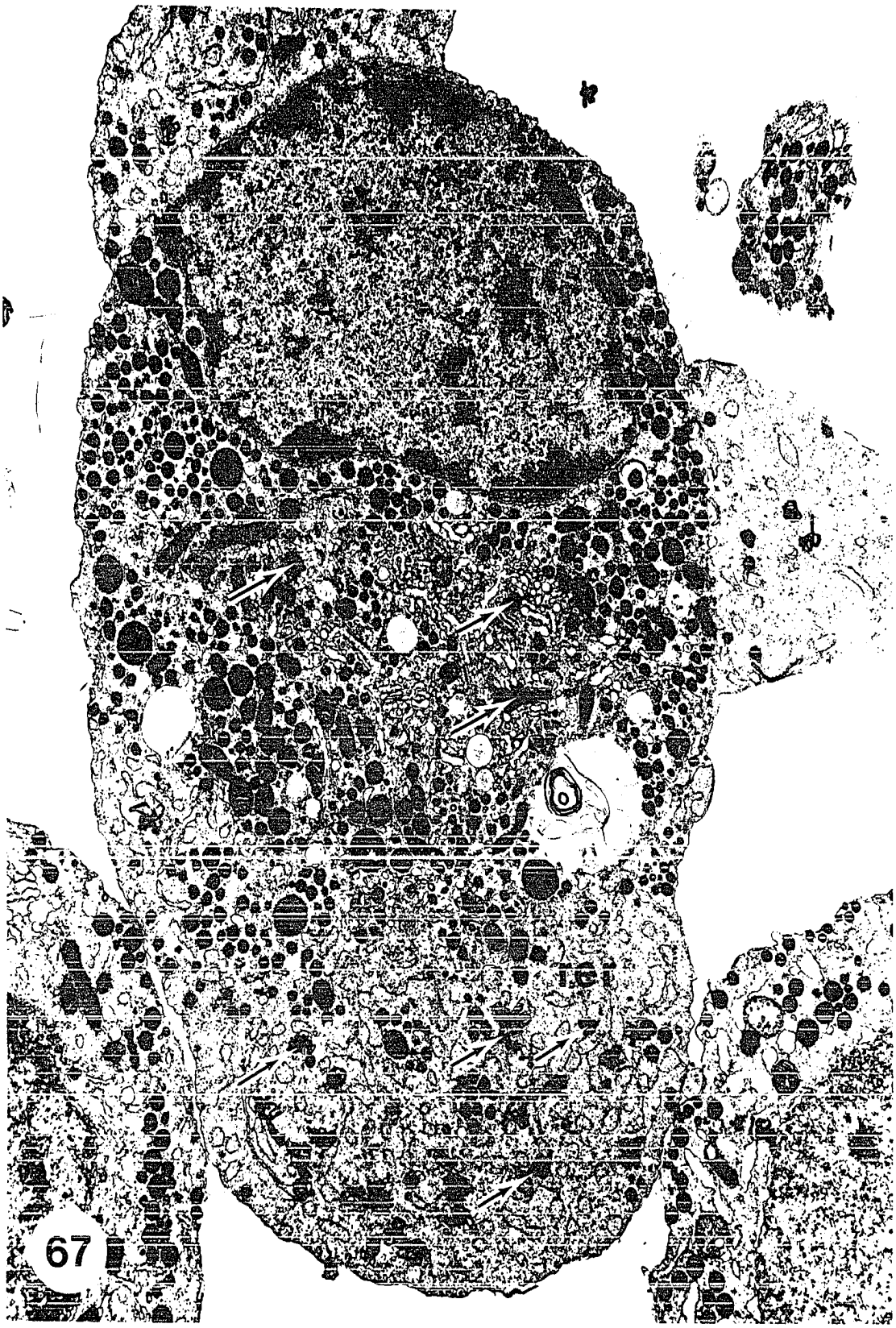
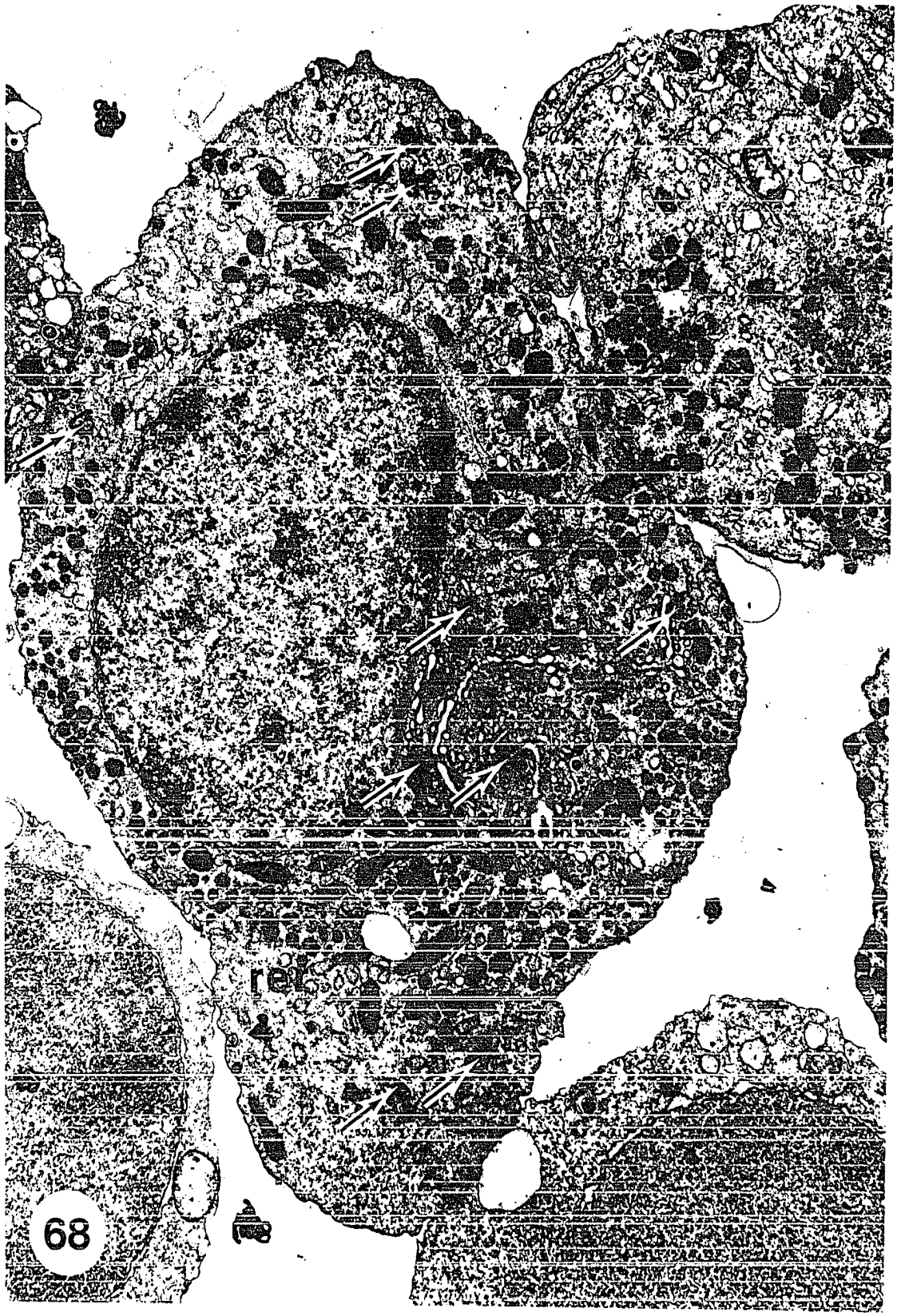

67

Figure 68. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 15 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


68

transitional elements of the cis face of the Golgi complex (Figure 69).

60 minutes One hour after the pulse, the greatest activity was in the Golgi complex (Figure 70). Silver grains were observed over outer, middle, and inner cisternae and over elements of the cis and trans faces of the Golgi complex (Figure 70). Activity over the RER had diminished.

120 and 240 minutes By this time the silver grains were over the immature and mature secretory granules (Figures 71 and 72). The remainder of the synthetic and secretory apparatus of the cell showed no activity.

Pulse labeling with mannose-^{3}H (30 days after castration)

0 minutes There was abundant labeling over the vast cisternae of the rough endoplasmic reticulum (Figure 73). There was no labeling over the Golgi complex, or the secretory granules.

5 minutes More silver grains were present over the dilated cisternae of the RER with some activity observed in the transitional vesicular elements of the SER (Figure 74).

15 minutes The silver grains were concentrated over the transitional elements of the SER on the cis face of the Golgi complex (Figure 75). There was little or no activity in the cisternae of the fenestrated trans elements in the Golgi complex.

30 minutes By this time the greatest number of

Figure 69. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 30 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

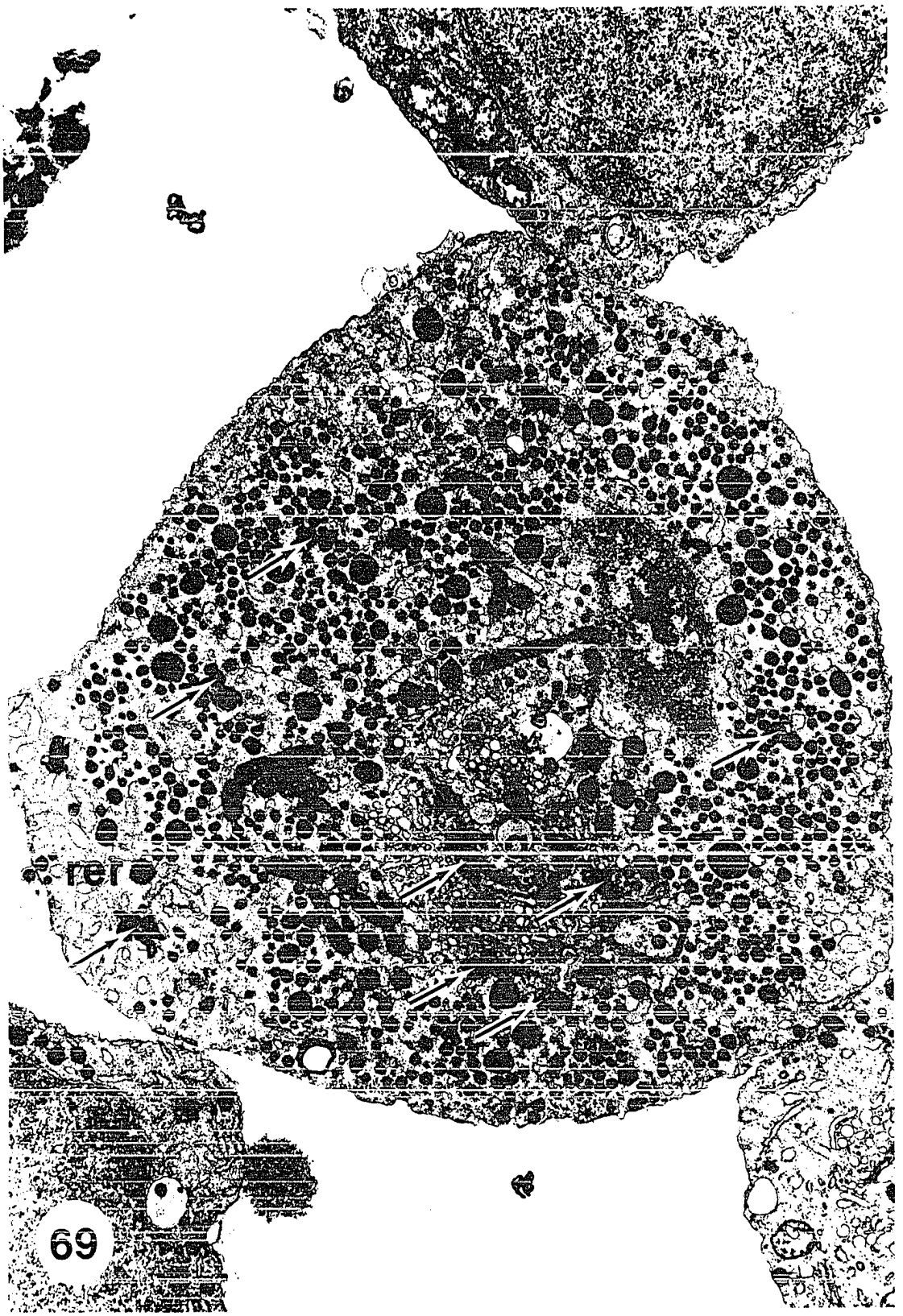

rer
69

Figure 70. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 60 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

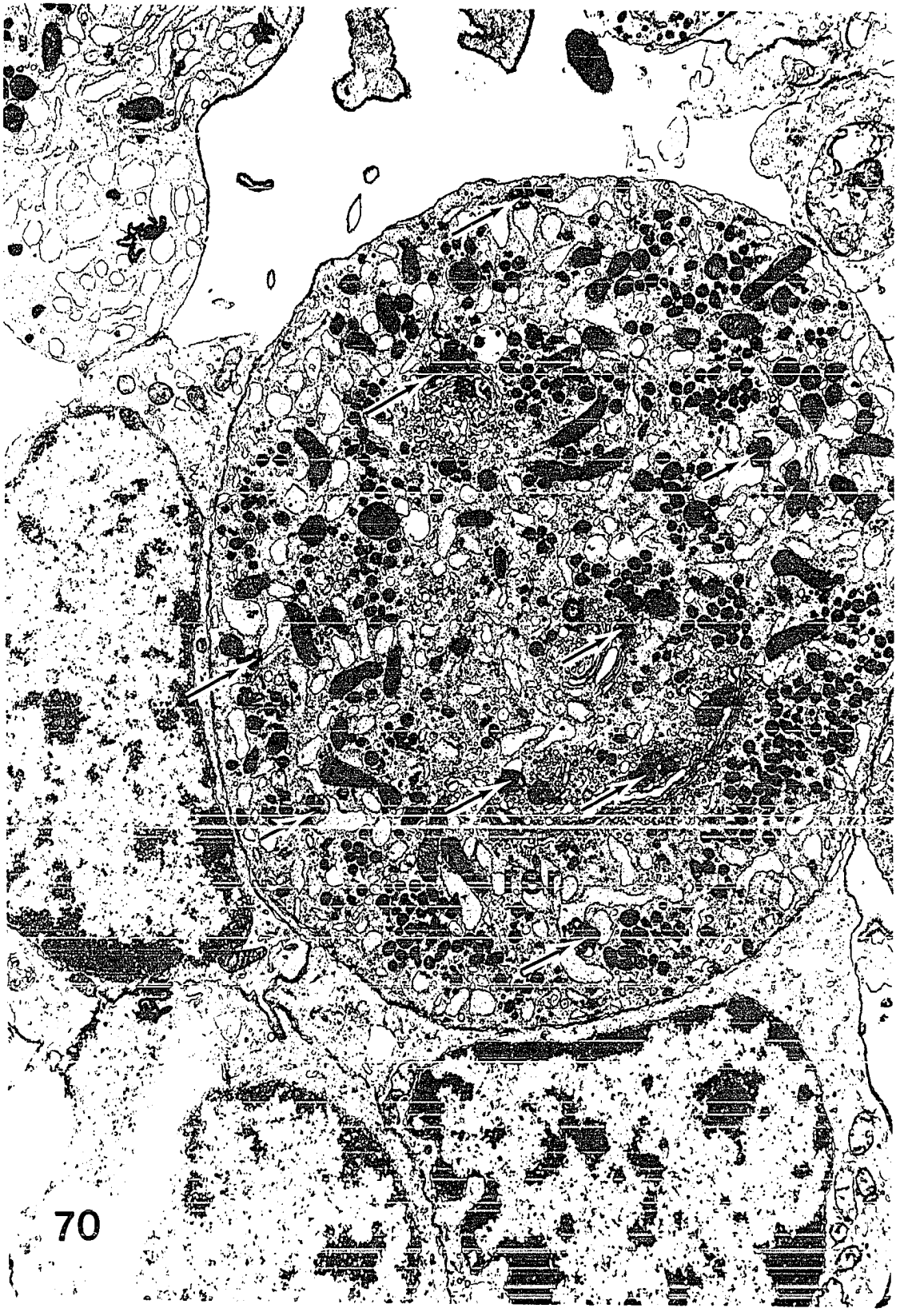

70

Figure 71. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 120 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

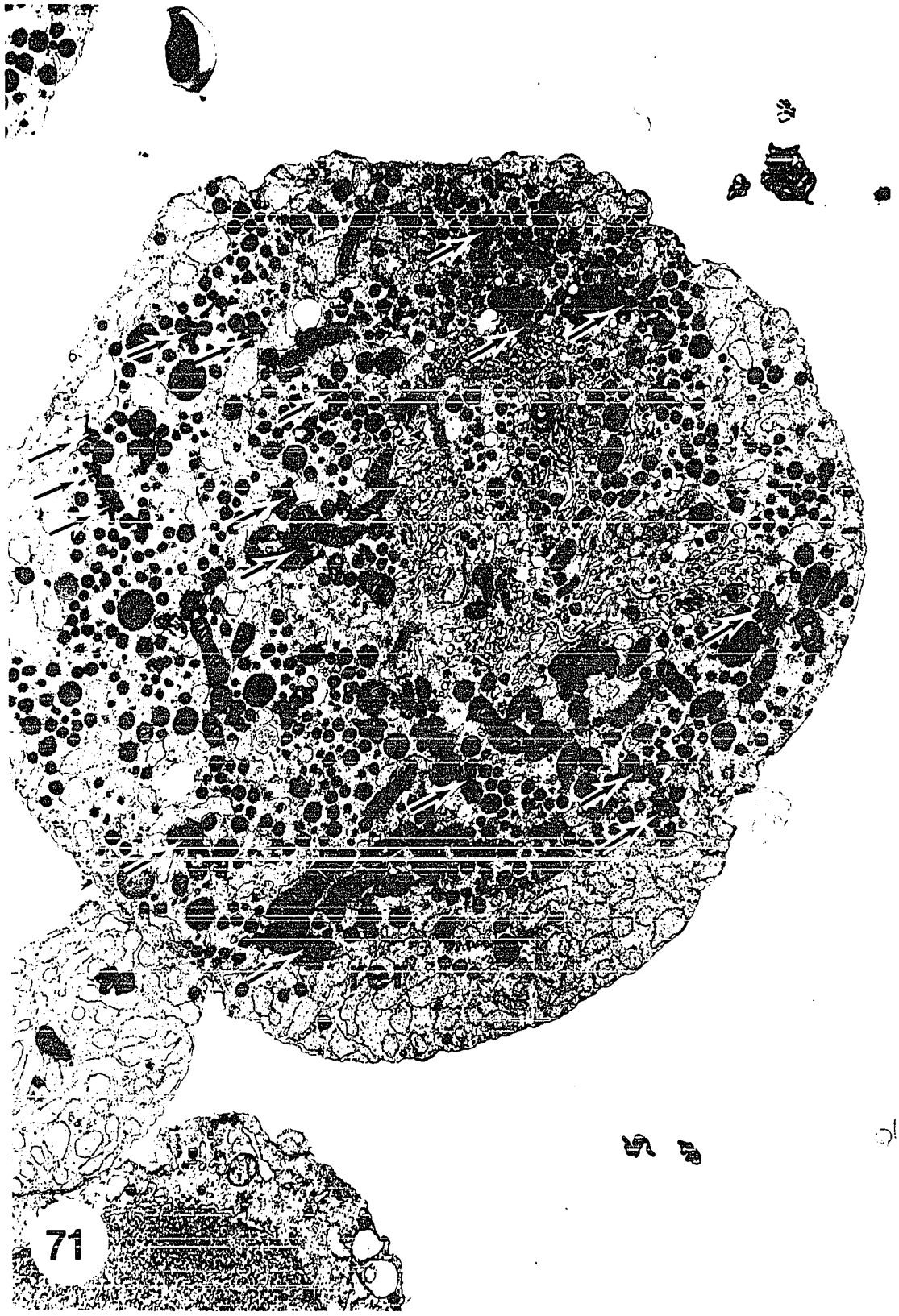

71

Figure 72. Autoradiograph of a gonadotroph from dissociated adenohypophyses 15 days after castration pulse labeled with mannose-^{3}H and incubated for 240 minutes, showing rough endoplasmic reticulum (rer). Arrows indicate silver grains. x11,500.


72

Figure 73. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 0 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

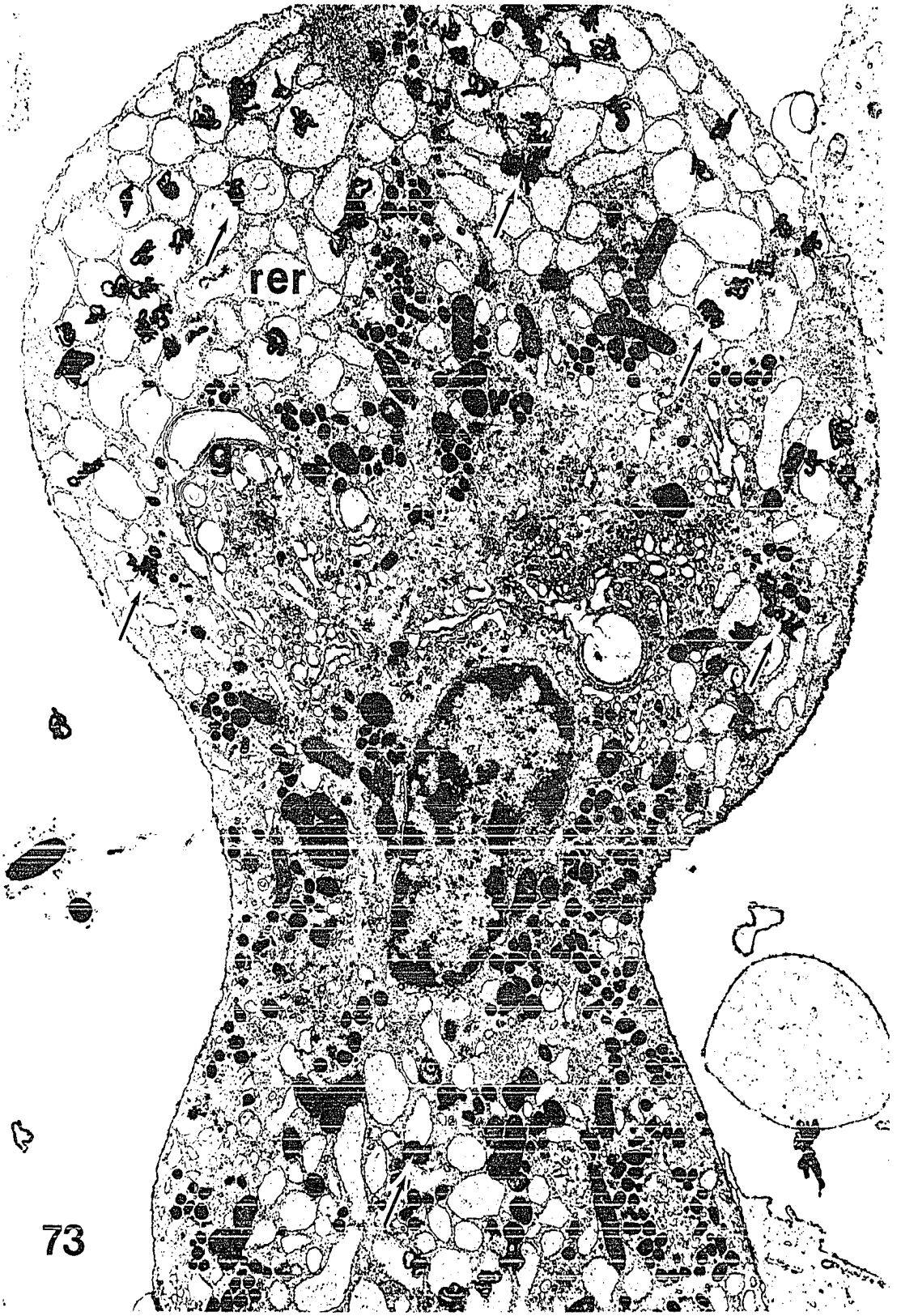
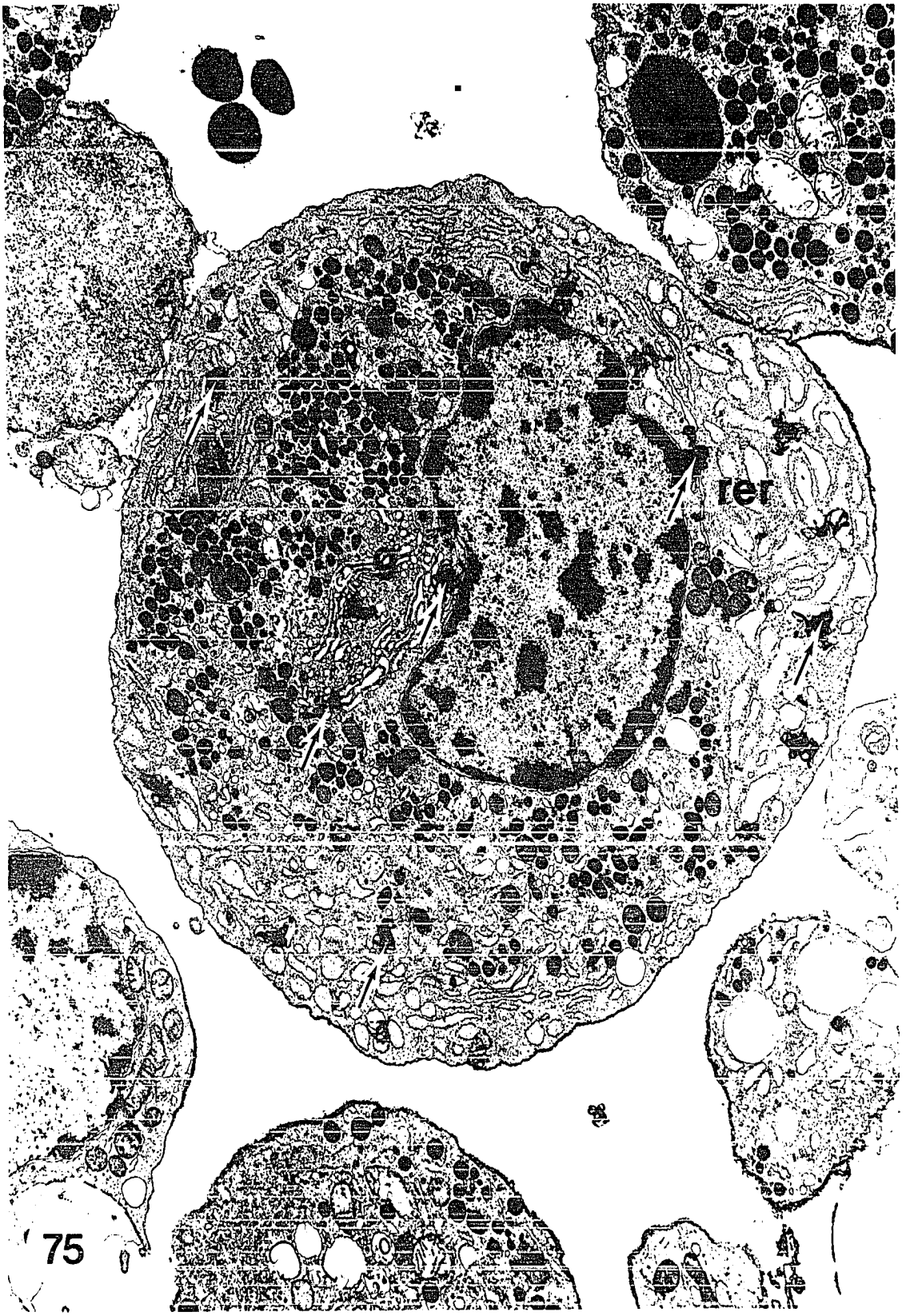

rer
g
73

Figure 74. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 5 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


rer
74

Figure 75. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 15 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


rer
75

silver grains was over the Golgi saccules and the immature secretory granules on the trans face of the Golgi complex (Figure 76). There were a few silver grains over mature secretory granules as well as the RER (Figure 76).

60 minutes The activity over the Golgi complex had peaked by one hour and the trans elements (both tubular and fenestrated), some of which contain immature secretory product, showed the greatest activity (Figure 77). However there were still some silver grains over the endoplasmic reticulum (Figure 77).

120 and 240 minutes By this time nearly all the activity was located in the mature secretory granules and the trans elements of the Golgi complex (Figures 78 and 79). Some silver grains did remain over the tubular and vesicular elements of the Golgi complex (Figures 78 and 79). The remainder of the cell was not active and only background numbers of silver grains were observed over the initial portions of the synthetic and secretory apparatus of the cell (Figures 78 and 79).

Figure 76. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 30 minutes showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

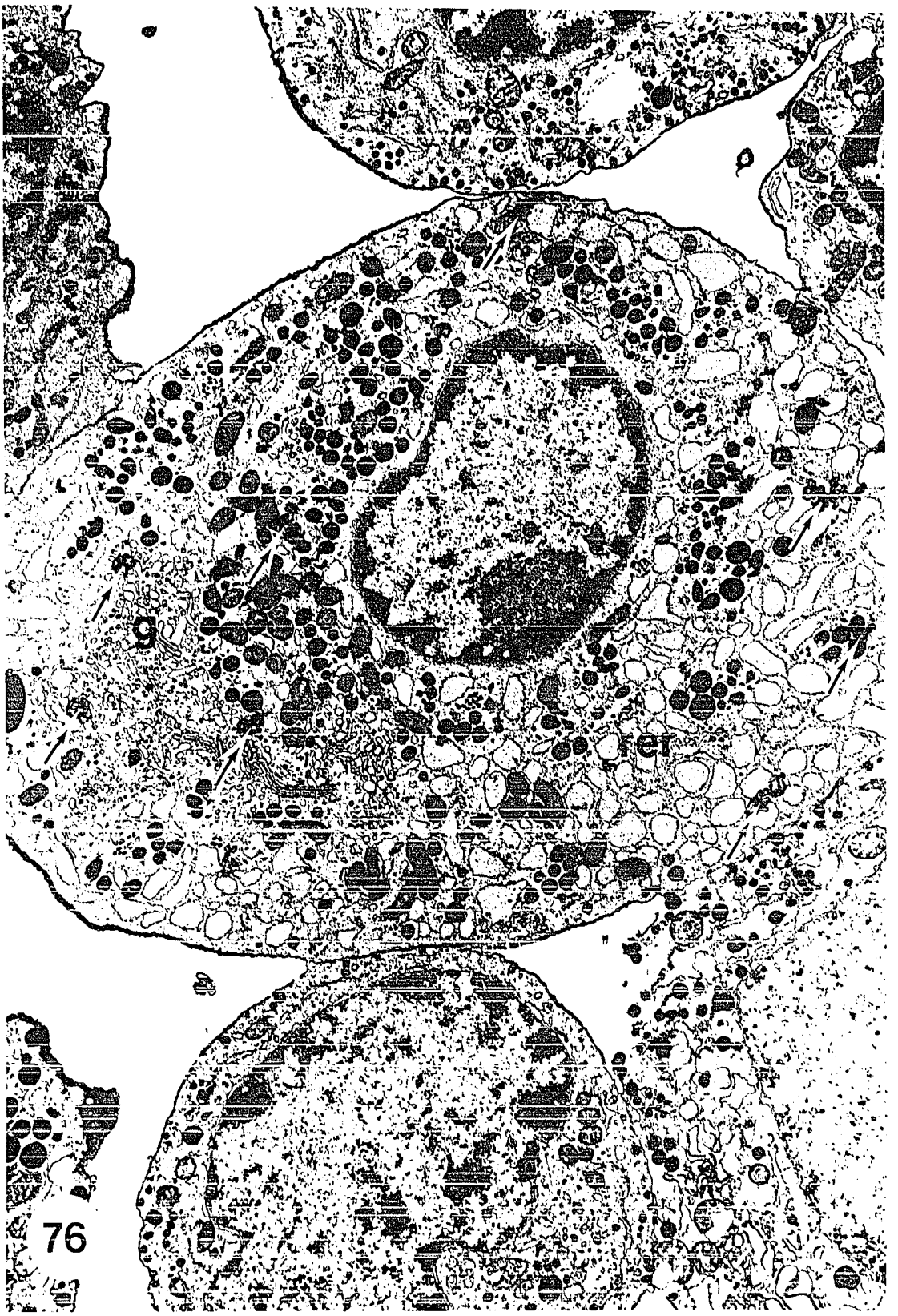

g
rer
76

Figure 77. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 60 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.


g
er
77

Figure 78. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 120 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

78

Figure 79. Autoradiograph of a gonadotroph from dissociated adenohypophyses 30 days after castration pulse labeled with mannose-^{3}H and incubated for 240 minutes, showing rough endoplasmic reticulum (rer) and Golgi complex (g). Arrows indicate silver grains. x11,500.

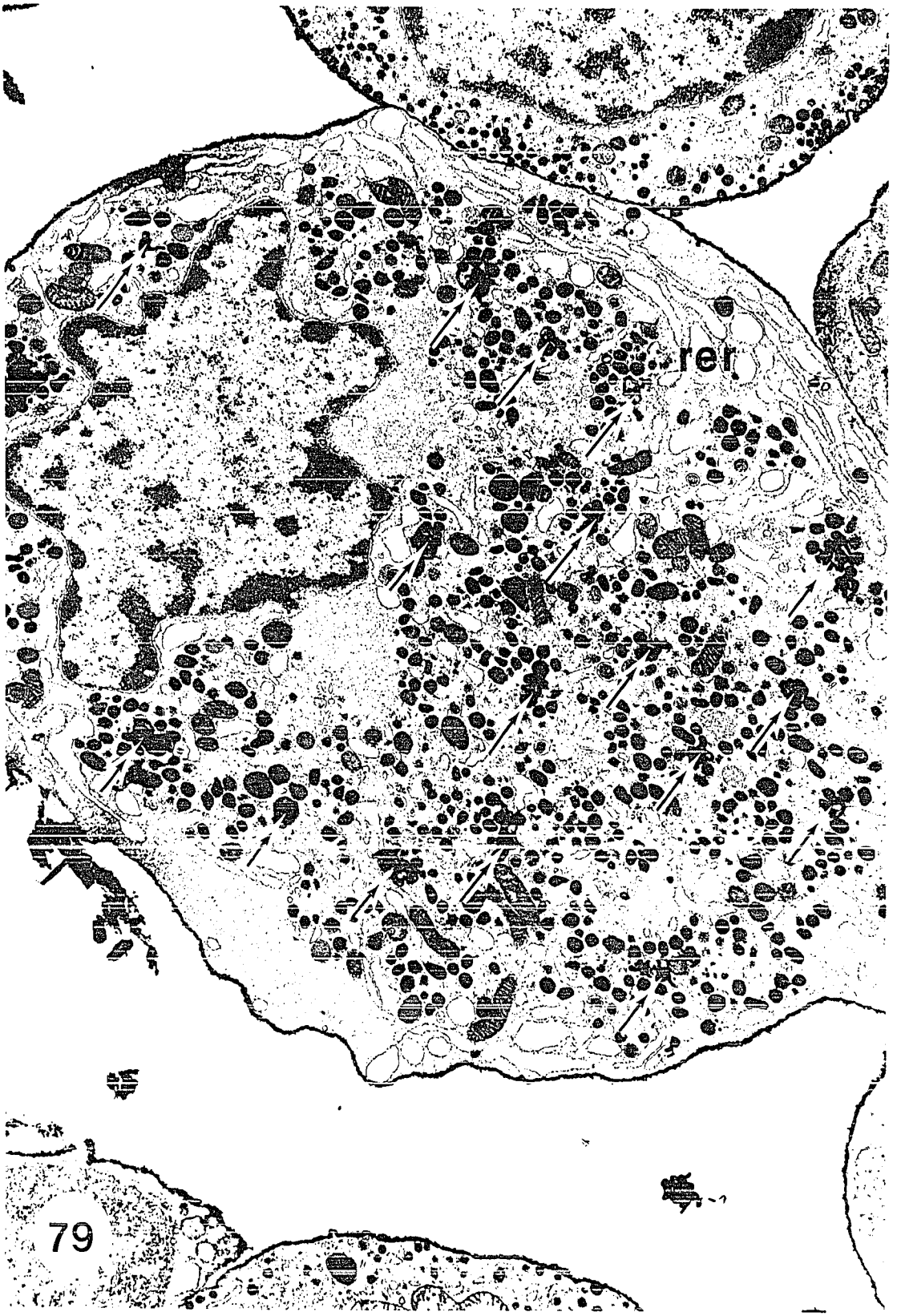
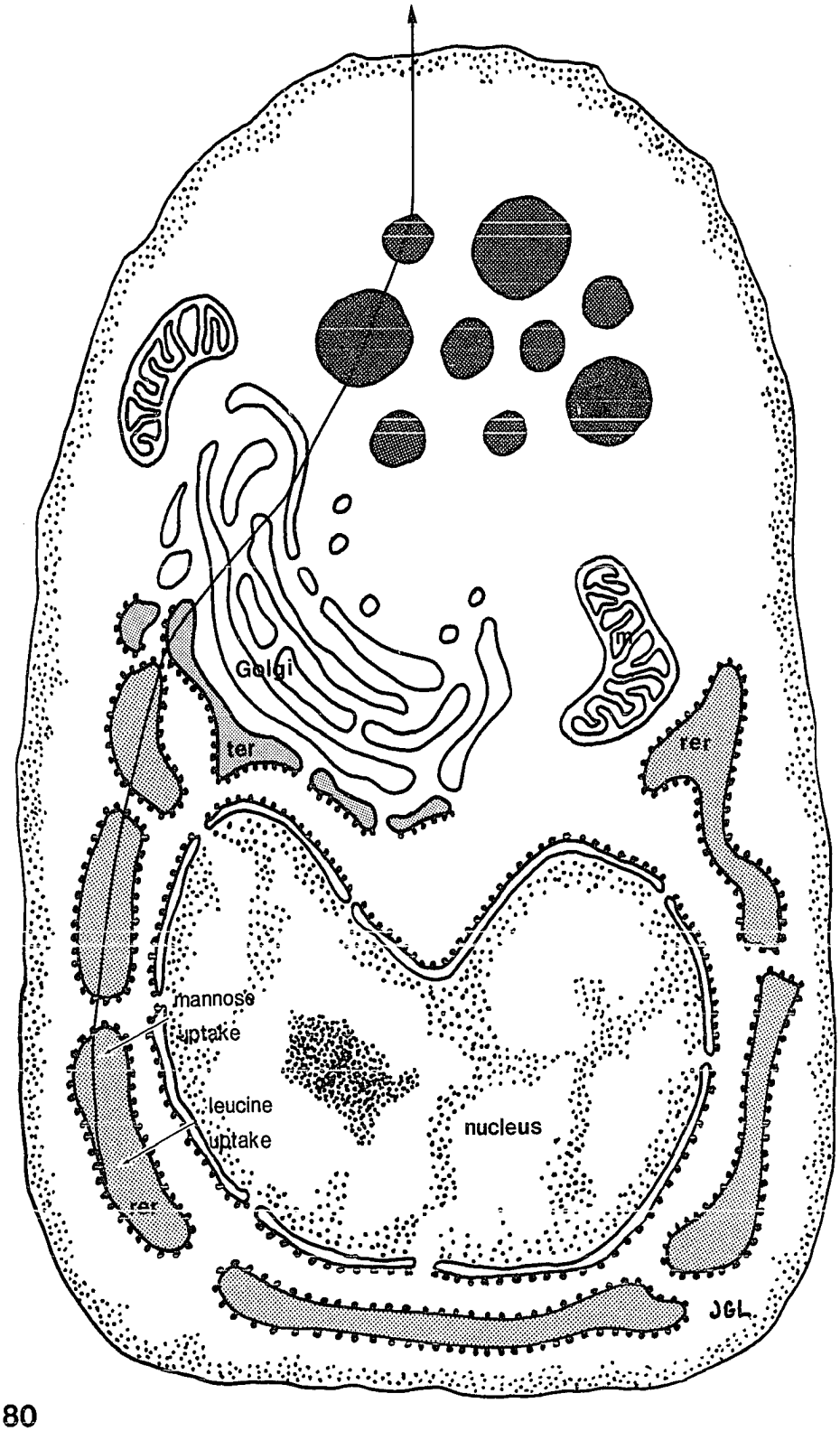

rer
79

Figure 80. Diagram of part of a gonadotroph cell of the adenohypophysis of a rat which illustrates the pathway taken by ICSH precursors from the time of synthesis of the polypeptide chains on the ribosomes of the rough endoplasmic reticulum (rer), as shown by the incorporation of leucine-^{3}H. Mannose-^{3}H is incorporated almost immediately afterward in the cisternae of the rough endoplasmic reticulum. By one hour the precursors have migrated through the transitional endoplasmic reticulum (ter) to the Golgi complex (Golgi), and after two hours they have moved to the secretory granules.


Golgi
ter
rer
mannose
uptake
leucine
uptake
nucleus
rer
JGL

80

DISCUSSION

Dissociation Techniques

Dissociation of male rat pituitary glands furnishes a new approach to *in vitro* analysis of secretory processes. The limitations encountered by the use of large pieces of pituitary tissue in the study of secretion at the subcellular level are avoided. This study has shown that dissociated pituitary cells are not significantly altered by this dissociation procedure and that physiologically altered "castration" cells are also unaltered by the dissociation procedure. The fine structure of these cells is well preserved if not enhanced by better penetration of the fixatives. These cells appear to be viable functioning secretory cells which provide enhanced material for physiological studies involving incorporation of labeled synthetic precursor materials.

The dissociation technique used in this study for the preparation of rat anterior pituitary cells is adapted from techniques previously reported by Hopkins and Farquhar (1973) and Portanova et al. (1970). Both of these techniques provided large numbers of dispersed cells. The functional and structural integrity of the cells prepared by these treatments is verified by electron microscopy which revealed isolated pituitary cells with intact plasma membranes and an abundant supply of subcellular organelles, including endoplasmic reticulum, Golgi complex and secretory granules.

Morphological identification of all the cell types was easily achieved and this was an important criterion for future experimentation.

Hopkins and Farquhar technique

This was the first method used in this study and gave very encouraging results. This technique is based on: (a) a short (15 minute) incubation in trypsin, followed by (b) a short (15 minute) incubation in neuraminadase-EDTA mixture, and, finally, (c) mechanical dispersion in a Pasteur pipette. All incubation media contained 0.3% BSA, 14 mM glucose and a complete amino acid supplement in a Krebs-Ringer bicarbonate solution (KRB). The use of neuraminadase was based on the fact that cell surface glycoproteins are known to be involved in cell adhesion (Ambrose, 1966; and Curtis, 1967) and this suggested that this enzyme (which acts on surface sialic acid groups) might be an agent useful for cell dissociation. The potential importance of such substances in adhesion of pituitary cells was also suggested by the fact that both cell junctions and the connective tissue framework are modest in the anterior pituitary gland as compared to many other organs. As far as junctions are concerned, the total area of cell surfaces devoted to them is limited in anterior pituitary cells compared to the situation encountered in the liver, exocrine pancreas, thyroid gland, intestine, and other lumen-lining epithelia (Farquhar et al., 1975).

Only shallow gap junctions and occasional desmosomes connect secretory elements to one another (or secretory to nonsecretory). The only other junctions encountered are those present between nonsecretory elements (follicular cells and endothelial cells).

Electron microscopy demonstrated that the fine structure of cells obtained from the pituitaries of normal and castrated rats by the above dissociation procedure was well preserved. It is clear that the rounded-up, single cells have certain advantages over their counterparts in intact pituitary tissue. Their main advantage is that they represent a functionally homogeneous cell population with equal access to metabolites, tracers, O_2, etc. In addition they have certain secondary advantages in that they present a favorable geometry with no long processes or pseudopods, a high incidence of cell profiles including all cell compartments of interest for the secretory process which enhances autoradiographic analyses.

A number of successful attempts have been made to produce viable single cell suspensions from a variety of tissues, e.g., liver (Howard et al., 1967; Berry and Friend, 1969), adrenal gland (Sayers et al., 1971; Halkerston et al., 1968; Swallow and Sayers, 1969), corpus luteum (Gospodarowicz and Gospodarowicz, 1972), thyroid gland (Maayan and Ingbar, 1968) and exocrine pancreas (Amsterdam and Jamieson, 1972) including the anterior pituitary (Portanova et al., 1970;

Hymer and Evans, 1970; Bala et al., 1970; Sayers et al., 1971; Vale et al., 1972; Ishikawa, 1969; Leavitt et al., 1973; and Hymer et al., 1973). Most of these procedures have involved the use of collagenase, hyaluronidase, trypsin, pronase, EDTA, and calcium-free media, alone or in various combinations. Hopkins and Farquhar (1973) were the first to use neuraminadase as a dissociating agent.

Initial treatment with protease was found to be essential for cell dissociation and this is in agreement with a study by Amsterdam and Jamieson (1972). Crude proteolytic enzyme extracts or proteases other than trypsin, e.g., Viokase (Vale et al., 1972) and pronase (Leavitt et al., 1973) have been used for pituitary cell dispersion but none has the dual advantage of being well-characterized and of having available a selective inhibitor (such as SBTI) which is effective under physiological conditions. This is important in the light of a number of reports which have emphasized the importance of specifically inhibiting protease activity, since these enzymes, which are known to adsorb to the cell surface and survive conventional washing procedures (Barnard et al., 1969; Wallis et al., 1969; and Poste, 1971) can cause injury to the cell surface and prevent reappearance of the cell coat material removed by enzyme action even at very low concentrations (Barnard et al., 1969; and Poste, 1971). However, when protease treatment is brief and efficiently terminated with appropriate inhibitors, removal of cell coat material is

reduced and resynthesis and replacement of these cell surface components occurs in hours (Zajac and Crowell, 1965; Schwartz and Nathenson, 1971; and Kono, 1969).

The probable effect of neuraminadase as a dissociating agent was suggested by indications that sialic acid groups are involved in cell adhesion (Ambrose, 1966; and Curtis, 1967) and reports that the specific enzymic removal of these groups decreases cell aggregation (Weiss, 1961; and Kemp, 1970). The rationale for this procedure is that anterior pituitary cells have few or at least limited areas devoted to intercellular junctions of any kind and a limited connective tissue stroma, so that cell adhesion must depend primarily on properties of the cell coat, or more precisely, on the surface glycoproteins. Quantitative results on other cell types (McQuiddy and Lilien, 1971; Codington et al., 1970; and Cuatracasas and Illiano, 1971) suggest that removal of sialic acid groups can be accomplished by prolonged tryptic digestion or brief, sequential trypsin-neuraminadase treatment. Thus, the neuraminadase allows the trypsin incubation to be shortened from 90 minutes in most cases to 15 minutes.

It should be noted, however, that neuraminadase was proven to be cytotoxic for pituitary cells at high concentrations (16 μg/ml) or after prolonged incubation (Hopkins and Farquhar, 1973). Furthermore, even at low concentrations and short intervals of exposure, this enzyme is known to affect

a number of important reactions at the cell surface (Marcus and Schwartz, 1968; Currie et al., 1968; Burger and Goldberg, 1967; Burger, 1969; and Rosenthall and Fain, 1971), including the damage of specific receptor sites in fat cells (Cuatracasas and Illiano, 1971; and Kraemer, 1966). Mouse tumor cells (Hughes et al., 1972) treated with neuraminadase rapidly (i.e., within 6-12 hours) replaced the sialic acid removed. It is further known that L-glutamine, an essential precursor in the synthesis of complex carbohydrates (White et al., 1968) is required for this process to take place.

Portanova, Smith and Sayers technique

This method of preparing dispersed pituitary cells was tried in an effort to simplify our procedure and for economy. The technique is based on: (a) mechanical agitation of tissue in trypsin for 60 minutes, followed by (b) treatment with lima bean trypsin inhibitor. All of the media were gassed with 95% O_2 and 5% CO_2 and contained 3% bovine serum albumin with a complete amino acid supplement. This technique is similar to that reported by Swallow and Sayers (1969) for the preparation of isolated cells from rat adrenal cortex. Mechanical agitation of pituitary tissue is very effective in dispersing large numbers of pituitary cells.

The functional integrity of trypsin dissociated cells from the anterior pituitary has been demonstrated previously in several ways. These dissociated pituitary cells have been

shown to contain large amounts of growth hormone and also relatively large amounts of ACTH, a very small fraction of which escapes into the medium upon incubation (Molamed et al., 1971). Extracts of hypothalamic-median eminence tissue caused release of ACTH when added to the cell suspension (Molamed et al., 1971). The release of hormone is inhibited when calcium ion or glucose is omitted from the incubation medium. These findings indicated that trypsin dissociated cells were intact and functional. Observation of these cells by Molamed et al. (1971) and myself with the electron microscope showed that trypsin dissociated cells are basically intact and undamaged. These cells were indistinguishable from those obtained using the Hopkins and Farquhar (1973) technique.

Final dissociation technique

To enhance cellular preservation and function over a 4-hour incubation period, some features of both dissociation techniques were employed. Essentially the techniques of Portanova et al. (1970) were used. In addition, glutamine was added to a concentration of 2 mM in the incubation media to augment resynthesis of cell surface glycoproteins. The media was gassed for 10 minutes with 95% O_2 and 5% CO_2 prior to use. All of the glassware was siliconized. The cells were removed at 10-minute (rather than 20-minute) intervals. Two ml of rat serum were added to the dissociated cells to

facilitate inhibition of trypsin after each 10 ml aliquot of cells was collected. Trypsin inhibitor was added to the cells when they were resuspended following centrifugation. All labeling and subsequent chase incubation media contained equal volumes of KRB and minimal Eagle's medium. The final incubation solution was minimal Eagle's medium (pH 7.4) with 15% rat serum and 2 mM glutamine. In experiments with mannose-^{3}H, sodium pyruvate was added to all media to a concentration of 10 mM to reduce the conversion of mannose into amino acids. An electron microscopic comparison of cells (normal or castrated) from intact pituitary tissue with those obtained using the dissociation technique above (Figures 1-37) show that we indeed have viable, well-preserved cells, with a normal complement of organelles. All five secretory cell types are easily identified and the two nonsecretory elements are present (Figures 1-37).

Intracellular Incorporation and Migration of Leucine-^{3}H and Mannose-^{3}H

Electron microscopic autoradiography was used to establish the intracellular locations by elucidating sites where leucine and mannose were incorporated into interstitial cell stimulating hormone (ICSH) or follicle stimulating hormone (FSH), under normal and stimulated (castration) conditions. This was done by locating the label at very early time intervals (0-5 minutes after the pulse) to minimize the chance

of subsequent migration. The second purpose of this experiment was to identify the chronological pathway of the labels from their sites of incorporation to their final destination in the secretory granules.

Interstitial cell stimulating hormone (ICSH) is a glycoprotein; this was confirmed by Ward et al. (1959). The polypeptide portion of this compound is divided into two subunits, α and β, and it is the β subunit which endows the ICSH molecule with its distinguishing biological activity. The α subunit is similar or identical to the α subunits of thyroid stimulating hormone and follicle stimulating hormone (Liu et al., 1970). The α subunit has two points of attachment of carbohydrate moieties to asparagine residues in the number 56 and 82 positions near the COOH-terminal end of the molecule, while the β subunit has one asparagine residue (residue number 13) near the NH_2-terminal for the attachment of one carbohydrate moiety. There are two leucine residues in the α subunit and eleven leucine residues in the β subunit (Liu et al., 1971). The quantitative analysis of the carbohydrate components of ovine luteinizing hormone demonstrated the presence of 8 residues of N-acetylglucosamine, 3 residues of N-acetylgalactosamine, 7 residues of mannose, 2 residues of galactose, 1 residue of fucose and 0 to 1 residue of sialic acid per molecule (Walborg and Ward, 1963). Based on this information, leucine-^3H and mannose-^3H were used to study the synthesis and transport of ICSH in the gonadotrophic cells of

the rat adenohypophysis.

Leucine-^{3}H Incorporation

In the case of leucine ^{3}H at the earliest times of incubation, all the radioactivity in the cytoplasm was located in the RER, in both normal and stimulated cells (Figures 38, 39, 45, 46, 53, and 67), which is therefore considered to be the site of incorporation of leucine into ICSH. Work on the pancreatic exocrine cell has revealed that secretory proteins, upon release from attached ribosomes, are subjected to a number of intracellular transport operations (Jamieson and Palade, 1967a,b). The initial step in the sequence is the vectoral transport of newly synthesized polypeptides to the cisternal space of the RER (Jamieson and Palade, 1968a.b.). This step depends on chain termination and seems to be controlled primarily by structural restrictions at the ribosome-membrane junction (Jamieson and Palade, 1968a.b.).

In all specimens (normal and castrated) taken 15 minutes after the pulse, the radioactivity is on the cis face of the Golgi complex (Figures 40, 47 and 54) with even more activity evident after 30 minutes (Figures 41, 48 and 55). This is the second step in the sequence of processing this hormone through the gonadotroph, i.e., transport from RER and the cis face of the Golgi complex. By 60 minutes, the radioactive leucine is concentrated in the lamellae of the Golgi complex and a few silver grains are observed over secretory granules

(Figures 42, 49 and 56). There is also a great deal of activity in the peripheral elements of the Golgi complex (Figures 42, 49 and 56). Jamieson and Palade (1968a.b.) found this step in the secretory sequence to be dependent upon ATP generated by oxidative phosphorylation. In the absence of this energy source, transfer is blocked on the cis side of the small peripheral vesicles of the Golgi complex. Apparently the chain of compartments involved in intracellular transport is provided with a lock at this level whose function is energy dependent. At present, it is thought that this energy is used to connect the RER cisternal space with that of the secretory granules, and the Golgi complex participates in the connection. By 120 minutes the hormone storage granules of both normal and castration cells contain nearly all of the radioactivity and by 240 minutes, some of the radioactive leucine may have been lost due to exocytosis of the secretory granules (Figures 43, 44, 50, 51, 57 and 58).

Mannose-^{3}H incorporation

At the earliest times of incubation (0 minutes) all of the radioactivity was present over the RER (Figures 59, 66 and 73). This may thus be considered the site of incorporation of mannose-^{3}H into ICSH precursors. Because of the limitations of resolution, it could not be demonstrated conclusively whether mannose incorporation took place on the polysomes or in the cisternae. Synthesis of the protein is

restricted to the ribosomes in association with the endoplasmic reticulum, whereas sugar groups are added at different sites in the cell. The sugar groups closely linked to the polypeptide chain (often including glucosamine, galactosamine and mannose) are added either as the presumptive polypeptide chains come off the ribosomes or in the cisternae of the ER while the more terminal sugars (often including galactose, fucose, and sialic acid) appear to be added in the Golgi complex. Nevertheless, the label is located over the cisternae of both normals and castrates by five minutes (Figures 60, 67 and 74). By 15 minutes post-pulse, the label is on the cis side of the Golgi complex (Figures 61, 68 and 75) and by 30 minutes mannose-^{3}H is in the cis vesicular and fenestrated elements of the Golgi complex (Figures 62, 69 and 76). Thus there is a gradual decline of grain deposition over the RER and a concomitant increase of activity over the Golgi complex. By 60 minutes, activity in the Golgi complex of normal and castrated rats has peaked (Figures 63, 70 and 77) and there is little or no activity over the RER. Two hours after the initial pulse, there is activity over the mature secretory granules (Figures 64, 71 and 78) and by four hours the secretory granules are heavily labeled (Figures 65, 72 and 79) with no activity in the preceding secretory compartments.

The migratory pathway described for mannose-^{3}H label, that is RER-Golgi-secretory granules, corresponds to the

pathway of the polypeptide chain of ICSH, as shown by labeling with leucine-^{3}H. Biochemically, it has been shown in thyroid tissue that mannose-^{14}C labeled the microsomes, but not their polysome fraction (Boughillous and Cheftel, 1966). Whur et al. (1969) have incubated lobes of rat thyroid tissue pulse labeled with mannose-^{3}H, galactose-^{3}H or leucine-^{3}H and studied autoradiographic silver grain distribution. They found that mannose-^{3}H localizes initially in the RER, and by 1-2 hours much of the radioactivity is transferred to the Golgi complex. At 3 hours they report significant grain deposition over apical vesicles and colloid. Hence, it seems most likely that the incorporation of mannose in this experiment took place within the cisternae after completion of polypeptide synthesis. It may thus be concluded that the mannose label migrated with the polypeptide chains and, therefore, was attached to them.

Glycoprotein Synthesis

Possible biochemical pathways

The peptide components of glycoproteins may be released from their sites of synthesis on ribosomes prior to the addition of carbohydrate. This has been verified by experiments utilizing ^{14}C labeled sugars and amino acids in the study of their incorporation into liver microsomal glycoprotein (Sarcione et al., 1964). In this study the site of incorporation for leucine-^{3}H was determined to be on the membranes

or in the cisternae of the RER (Figures 38, 39, 45, 46, 52 and 53). As the polypeptide is synthesized on the ribosome, it moves through a groove in the large ribosomal subunit into the cisternae of the RER (Molnar, 1975). During the elongation of the polypeptide, it has been proposed that membrane bound glycosyl transferases link an N-acetylglucosamine to an asparagine residue in the polypeptide chain as it enters the RER cisternae.

At this point, two possibilities are postulated in terms of oligosaccharide elongation: (a) the first envisages the successive direct step-wise transfer of monosaccharides from corresponding sugar nucleotides to the growing oligosaccharide chain (Schachter, 1973), or (b) as shown by recent work from the laboratory of L. F. Leloir (Behrens et al., 1973; and Levy et al., 1974) as well as from other laboratories, including those of E. C. Heath (Hsu et al., 1974), J. L. Strominger (Herscovics et al., 1974; and Wedgewood et al., 1974) and R. W. Jeanloz (Tkacz et al., 1974), there is a mechanism in animals and plants analogous to that involved in the synthesis of polysaccharides in bacteria, i.e., via glycosylated lipid intermediates. The nature of the lipid has not yet been conclusively established, but it is generally believed to be a dolichol phosphate. Dolichol is the name of a family of polyprenols containing from 17 to 22 isoprene units, common to many tissues. The dolichols were first discovered in mammalian tissues more than fifteen

years ago (Burgos et al., 1963). The dolichols have some of the largest molecular weights among the aliphatic compounds found in nature; they are very long and also have a saturated terminal isoprene unit bearing a hydroxyl unit, thus providing a stable site for phosphate linkage to dolichol. Most of the evidence accumulated to date concerning dolichol-linked sugars deals with the structure and biosynthesis of mannosyl phosphoryl dolichol. Hemming (1974) has provided a description of this compound and its possible role in glycoprotein synthesis. The sugars that have been shown to participate in the transfer reaction of a single sugar from a nucleotide to a lipid are mannose, N-acetylglucosamine and glucose.

In addition to single sugar transfer, information from the incubation of rat liver microsomes indicates that dolichol-pp-oligosaccharides containing N-acetylglucosamine terminals and many (3 to 16) mannose units are present (Hemming, 1974). The mannose oligosaccharide described above has a structure that is found in the "core" of many glycoproteins (Lennarz, 1975). It seems that this "core" mannose oligosaccharide moiety is transferred to the acceptor protein en bloc as a preasembled unit rather than a step-wise transfer of single sugars from lipid or nucleotide donors (Molnar, 1975).

Studies in the liver (Behrens et al., 1973), mouse myeloma tissue (Hsu et al., 1974) and chicken oviduct systems (Lucas et al., 1975) have shown that isolated, partially purified mannose-^{14}C-oligosaccharide-lipid serves

as the donor of the oligosaccharide chain to glycoprotein. Findings by Lucas et al. (1975) show that both labels in the oligosaccharide-lipid, which contains mannose-^{14}C and N-acetylglucosamine-^{3}H, are transferred to glycoprotein at a constant ratio and both labels were found in a constant ratio in the partially resolved glycopeptide fragments produced by proteolytic digestion of the enzymatically labeled glycoproteins. These findings provide strong evidence that en bloc transfer of the oligosaccharide chain from oligosaccharide-lipid to protein occurs.

The structure of the oligosaccharide is of particular interest because of its similarity to the core structure of a variety of soluble secretory proteins. This core structure terminates in a chitobiosyl unit, in which the terminal N-acetylglucosamine residue is linked to an asparaginyl residue. In many soluble glycoproteins (Huang and Montgomery, 1972; Kabasawa and Hirs, 1972; Lee and Scocca, 1972) as well as in one membrane glycoprotein (Kawasaki et al., 1974), the first mannosyl residue is linked to the interior N-acetylglucosaminyl residue by a β-linkage, whereas the more distal five or more mannose residues are linked by α-glycosidic bonds. The configuration of this core oligosaccharide, i.e., with five or more mannose residues and its position on the polypeptide chain, i.e., attached to an asparagine residue straightaway, helps to explain the profuse numbers of silver grains observed in this study over RER membranes and

cisternae 0 to 5 minutes after mannose-^{3}H incorporation (Figures 38, 39, 45, 46, 52 and 53).

Other glycosyl moieties, i.e., N-acetylglucosamine, galactose, fucose and sialic acid, are added to the core of the glycoprotein by glycosyltransferases in the transitional portions of the ER and the Golgi complex. This is a separate process by which single sugars are sequentially transferred from their respective nucleotide derivatives to the distal portion of incomplete oligosaccharide chains of glycoproteins. This is where the terminal residues are added and it is these terminal sugars that determine the differentiating character of each specific glycoprotein.

What is the reason for the existence of a process involving lipid linked intermediates in the assembly of the core oligosaccharide chains? A study of the subcellular distribution of the enzymes involved in the assembly of oligosaccaride-lipid and in the transfer of oligosaccharide chain to polypeptide may provide some answers. The available information suggests that highest activities are found in the endoplasmic reticulum (Dallner et al., 1972; Richards and Hemming, 1974; and Hsu et al., 1974). On the basis of this information, one working hypothesis is that the lipid-mediated assembly process functions in a step in which nascent proteins already associated with membranous components of the cell are converted to glycoproteins by the addition of the core oligosaccharide. Also the problem of sugar nucleotide penetration through the permeability barrier of the

cell membrane is obviated because a sugar linked to a lipid can readily diffuse to either surface of a membrane and thus be readily available as a substrate in proximal glycosylation of polypeptides. Probably one of the most interesting current aspects concerning the role of dolichol phosphates in glycosyl transfer is the potential they offer for controlling the rate of the glycosylation process.

It was postulated (Hemming, 1974) that the rate of glycosyl transfer in bacteria may be controlled by adjustment of the level of polyprenol monophosphate in the system and that at least in some bacteria this might be achieved not only through changing the rate of its biosynthesis but also by altering the balance between polyprenol monophosphate phosphatase and polyprenol phosphokinase. It is possible that a similar method of control could operate in mammalian cells.

Two possible phases of glycoprotein synthesis have been discussed. In the first phase, the oligosaccharide core is transferred from a lipid (dolichol) carrier to a protein. In the second phase, the additional terminal sugars are added in a stepwise fashion from their respective sugar nucleotide to the core oligosaccharide of the glycoprotein.

A third phase in this process may be considered to be the final disposition of the glycoprotein; i.e., will it be secreted or will it remain associated with cell plasma membrane? The determining factor here may be the hydrophobicity of a portion of the polypeptide chain.

Assuming that the mechanism of synthesis of the oligosaccharide chain proves to be the same for secretory and membrane glycoproteins, one can speculate that the overall process for both types of glycoprotein may proceed by the sequence proposed for secretory glycoproteins (Palade, 1975).

Perhaps during, or immediately following, translation of polypeptide chains in the RER, addition of the core oligosaccharide occurs via the lipid intermediate. The complete glycoproteins destined to be secreted have little or no affinity for the membrane and thus are released into the cisternal space; the membrane glycoproteins remain associated with the membrane of the RER. Next, the secretory glycoproteins, by a transport process, and the membrane proteins by lateral diffusion, are relocated in the Golgi complex. Here, terminal glycosylation of the core oligosaccharide of both types of glycoproteins occurs via glycosyl transferases and sugar nucleotides. Finally, secretory vesicles from the trans face of the Golgi complex fuse with the plasma membrane. The glycoproteins are subsequently released and the membrane glycoproteins become constituents of the plasma membrane. In this scheme proposed by Waechter and Lennarz (1976), the primary determinant of the final disposition of the two classes of glycoproteins is their primary and secondary structure, which determines their hydrophobicity and hence their affinity for membranes. Within the secretory system, several membranous components can be distinguished, together forming

the pathway along which the secretory product moves through the cell.

Ultrastructural considerations

The RER, the site of synthesis of secretory protein, is the compartment in which the first phases of secretion take place. This was shown to be the initial site of leucine-^{3}H and mannose-^{3}H incorporation within 0 to 5 minutes after a 5-minute pulse label with each of these precursor molecules. This study has shown that secretory glycoprotein, after having left the RER cisternae, appears to enter the Golgi complex within 30 minutes to one hour. Hence, some kind of connection between the membranes of the RER and the Golgi elements must exist, unless the secretory glycoprotein passes from the former to the latter by way of cytoplasmic matrix and there is no evidence for this type of transfer.

Caro and Palade (1964) observed cisternae of the RER adjacent to the cis face of the Golgi complex which are frequently devoid of ribosomes and called these "transitional elements" of the ER (Figure 80).

Two membranous structures associated with transitional endoplasmic reticulum (TER) are likely candidates for bringing about a connection between the cisternae of the RER and the Golgi elements. These are the tubular or sheetlike structures and the small (50 nm) vesicles described in most secretory cells (Zeigel and Dalton, 1962).

Possible relationships between the RER and the Golgi complex are, therefore, the following: (a) there is a permanently open connection as in a tubular system, (b) the connection is intermittently open as in a tubular system in which the continuity is broken and repaired by alternating processes of fission or fusion of its membranes, and (c) the connecting system is permanently discontinuous, as in a vesicular transport mechanism.

The next step is the transport of secretory glycoprotein from one side (cis) to the other (trans) of the Golgi complex and one can only speculate as to whether the exact transport involves flow of contents or movement of separate cisternae. In producing the secretory vesicles or granules, the Golgi complex sheds membrane. It is not known whether the replacing membrane is formed in the Golgi stack by membrane synthesis or by insertion of pre-existing pieces of membrane, or if it is added from the ER. The mechanism by which the glycoprotein content of the elements of the trans side of the stacks is concentrated is an open question, though it is accompanied by changes in membrane composition. As a result of changes in the membrane composition of the Golgi complex, the membrane of the secretory vesicle or granule acquires a chemical composition that resembles that of the plasma membrane (Morré et al., 1974). It also allows the granule to fuse with the cell membrane in the process called "exocytotic secretion." This was demonstrated by the work of Friedman

and Cardell (1972). The last portion of the secretory apparatus (secretory granule phase) was occupied by the labeled precursors two hours after the initial pulse and remained through four hours. The spatial relationship between the cis face of the Golgi complex and the ER, on the one hand, and between the trans Golgi cisternae and secretory granules, on the other, illustrates the key position the Golgi complex occupies in the secretory cell. In this study it was found to be the organelle controlling the rate of hormone processing through the secretory apparatus of the cell.

Conclusion

The dissociation technique used in this study provided viable and normally functioning populations of pituitary cells, both normal and castrated, which were capable of being incubated for up to 4 hours without visible deleterious effects. These cells were able to take up metabolic precursors, leucine-^{3}H and mannose-^{3}H, from the incubation media and process them through the secretory apparatus with no apparent difficulty. The synthesis of the glycoprotein ICSH appears to be consistent with the concept that these macromolecules pass from the RER to the Golgi complex in about one hour and then move into secretion granules by two hours. Mannose is apparently a core sugar and is thus added to the polypeptide backbone very early in the glycosylation process. The core oligosaccharide is rich in mannose residues and may

be added en bloc to the nascent polypeptide by transfer from an oligosaccharide-lipid (dolichol) donor. This transfer may provide a place for cellular control of glycoprotein synthetic rates. There appears to be no substantial change in the temporal sequence of these events upon stimulation of hormone synthesis. Nascent polypeptides accumulate within the cisternae of the RER but passage through the Golgi complex appears to limit the movement of hormone into greater numbers of secretory granules. The passage of ICSH through the cell is unidirectional with no reverse movement of radioactivity in either the normal or stimulated condition.

Summary

A modification of a dissociation procedure was developed for the anterior pituitary tissue which produced viable suspensions of single cells. The procedure involves incubation and mechanical agitation of small tissue blocks in 0.25% trypsin in KRB with a complete amino acid supplement, followed by treatment with 2.67% trypsin inhibitor in KRB with a complete amino acid supplement and 2 mM glutamine added. By electron microscopy all five types of secretory cells plus endothelial and follicular cells can be identified and are morphologically well-preserved up to 4 hours after dissociation.

The incorporation of leucine-^{3}H into ICSH begins within 5 minutes in the RER of the gonadotrophs after a 5-minute pulse label. This label moves to the next cellular

compartment, the Golgi complex, within 60 minutes. From here, it enters the secretory granules, the final compartment, within 2 hours.

Mannose-^{3}H follows the same route through the cell with a similar chronological sequence. It appears as though mannose residues comprise a major portion of the core of this glycoprotein. The core is an oligosaccharide attached to the protein and to which terminal sugar residues are added later. Dolichol, a hydrophobic lipid carrier molecule, may be an intermediate donor in the transfer of sugars to protein from nucleotides. They may also provide the en bloc transfer of entire core oligosaccharide chains in one step to a recently synthesized protein. The terminal sugars are added later, probably in the Golgi complex.

The ultrastructural relationships between the secretory compartments have been considered and they seem to support the pathway suggested above. The movement of labeled glycoprotein from the RER to the Golgi complex implies that some type of connection, whether intermittent or permanent, must exist between these two compartments. Movement through the Golgi complex is the next step and the details of this process are nebulous as are the specific mechanisms for concentration of secretory glycoprotein content into granules on the trans face of the Golgi complex.

The process of exocytotic secretion is the final phase in this scheme but no definitive explanation of granule

transport in gonadotroph cells to the plasma membrane is possible at this time.

Processing of secretory material seems to be guided to a great extent by the Golgi complex. This organelle occupies a key position in the secretory pathway. Movement of secretory material into and out of the Golgi complex requires energy, possibly for membrane fusion and fission, and it is within this structure that molecular character differentiating terminal sugar residues are selectively added to core oligosaccharides of both membrane bound and secretory glycoproteins. It is these glycoproteins that are potentially responsible for cellular binding sites, receptor sites and communication with other cells.

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